MITOCHONDRIAL-DNA VARIATION AND THE EVOLUTIONARY AFFINITIES OF THE *Peromyscus maniculatus* COMPLEX FROM WESTERN NORTH AMERICA

A Dissertation

by

MINDY LYNN WALKER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2005

Major Subject: Biology

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ABSTRACT

Mitochondrial-DNA Variation and the Evolutionary Affinities of the *Peromyscus maniculatus* Complex from Western North America. (December 2005)

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Chair of Advisory Committee: Dr. Ira F. Greenbaum

Intraspecific phylogeography and the phylogenetic relationships of recently-diverged taxa are best assessed with the use of a genetic marker that coalesces rapidly and thus provides phylogenetically informative characters for closely-related taxa. Mitochondrial DNA (mtDNA) fits these criteria and was thereby ideal for analyzing genetic variation within and among the youngest taxonomic members of the *Peromyscus maniculatus* species group, *P. sejugis* (restricted to two islands in the Sea of Cortés), *P. maniculatus* (distributed throughout North and Central America) and *P. keeni* (a coastal species restricted to the Pacific Northwest of North America). The approach utilized in this research involved sequencing a 1439 base-pair (bp) region of mtDNA for a total of 581 specimens representing 45 different geographic localities from along the west coast of North America. The sequences obtained were used to assess the partitioning of genetic diversity within and among these taxa, address phylogenetic and taxonomic concerns about the western representatives of the *P. maniculatus* species group and discuss the post-Pleistocene biogeography of the west coast of North America.

Analysis of mtDNA sequence variation, considered within the framework of a phylogenetic species concept, revealed the existence of two evolutionarily significant units of *P. sejugis* as well as a previously unrecognized sibling species nested within the Pacific coastal range of *P. maniculatus*. Moreover, analysis of intraspecific sequence divergence allowed for the identification of the ice-free refugium thought to harbor *P. keeni* throughout glaciation during the Pleistocene epoch. This work will establish the foundation for additional examination of cryptic genetic variation in different morphotypes of *P. maniculatus* and continue the precedent for recognizing *P. maniculatus*-group taxa that reflect true evolutionary entities.

To my family: Mom, Dad, Jeremy and Grandma for your strength and unwavering support.

And to Aric: you made me laugh every day and showed me that this is the way life is supposed to be.

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CHAPTER I

INTRODUCTION

The biodiversity and distribution of many of the extant species of North America has been primarily impacted by the major climatic and geologic events of the Pleistocene. This appears to be particularly relevant to the extant plants and terrestrial animals of the coastal Pacific region of North America. The overall goal of this research was a systematic and phylogeographic analysis of the deer mice (species in the *Peromyscus maniculatus* group) from western coastal North America and derivation of specific and general inferences relative to the major biogeographic patterns and processes that produced the current biodiversity of this geographic region.

The specific topics addressed included:

1) assess the taxonomic status of *Peromyscus sejugis* and address the question of whether the populations of *P. sejugis* are the result of fragmentation of an ancestral mainland population or dispersal followed by restricted gene flow due to isolation by distance
2) identify the geographic partitioning of haplogroups among populations of the *Peromyscus maniculatus* species group distributed from Baja California and along the west coast to Washington

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This dissertation follows the style and format of Molecular Biology and Evolution.

3) determine the relationship of these haplogroups to centrally-distributed *P. maniculatus* and to *P. keeni* in order to address heretofore unresolved phylogenetic issues and confirm or refute hypothesized polyphyly within *P. maniculatus*
4) assess the phylogeographic processes that have resulted in the present-day distribution of and genetic variation in *P. keeni*, a coastal species of the Pacific Northwest
5) ascertain the extent of geographical concordance (*sensu* Avise and Ball 1990) among co-distributed taxa in these coastal regions

Although co-distributed organisms are likely to be concordantly affected by large-scale vicariant events and formidable biogeographic boundaries, inferences relative to the effects of such large-scale historical climatic and geologic events on the evolution of biodiversity over broad geographic regions are best accomplished by analyses of terrestrial species distributed over the entirety of the geographic region in question. For coastal Pacific North America, the taxa in the *Peromyscus maniculatus* species group comprise the only mammals distributed across nearly the entirety of this geographic region extending from southern Baja California to southeastern Alaska and the southern Yukon Territory (Hall 1981). The ubiquitous occurrence of these mice over the Pacific coastal region and recent advances in analyses of genetic variation provide a unique opportunity to reconstruct their recent diversification, demography and phylogeography as a model for understanding the major process that produced the origin and partitioning of the biodiversity of the plants and terrestrial animals throughout this area.

Mice of the genus *Peromyscus* (Cricetidae, Neotominae) comprise the most successful group of small mammals endemic to North and Central America. The vast distribution and diversity of species and habitats in the genus have made it a preferred model organism for studies encompassing virtually all areas of organismal biology. In particular, members of this genus have played a central role in the development of systematic mammalogy in North America and have thus been favorably likened to *Drosophila* in their impact on the growth of systematic biology and evolutionary genetics as a whole (Carleton 1989).

Of the more than 50 species of *Peromyscus*, none is more widely distributed nor intensively studied than *P. maniculatus* and the closely related species in the *P. maniculatus* species group (Hall 1981). The monophyly of the *P. maniculatus* group and the systematic relationships of its major taxa (*P. maniculatus*, *P. polionotus*, and *P. melanotis*) are supported by morphologic, cytogenetic and molecular data (reviewed by Carleton 1989). Within this group, however, the phylogenetic relationships and evolutionary history of two peripherally distributed species, *P. sejugis* (Santa Cruz Island mouse, Dice 1940, Blair 1950, Hooper 1968, Carleton 1980, Hall 1981; endemic to Isla San Diego and Isla Santa Cruz in the Sea of Cortés) and *P. keeni* (Northwestern deer mouse, Hogan et al. 1993, Greenbaum 1999; coastal deciduous forests from the Olympic Peninsula of Washington to southeastern Alaska and southwestern Yukon), remain problematic. Allozymic (Avise et al. 1979) and chromosomal data (Gunn and Greenbaum 1986, Smith et al. 2000) reveal an unresolved trichotomy consisting of *P. keeni*, *P. sejugis* and *P. maniculatus* (Fig. 1a). Three alternate hypotheses (Fig. 1) can

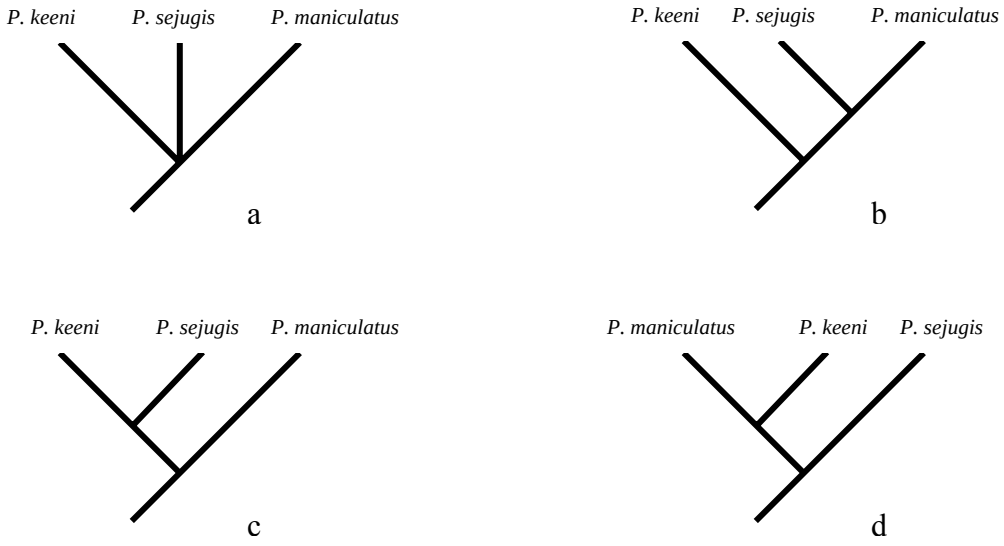


FIG. 1.–The unresolved trichotomy (a) of *P. keeni*, *P. sejugis* and *P. maniculatus* and alternative hypotheses indicating the potential phylogenetic relationships of these taxa.

be advanced to explain this trichotomy: 1) *P. sejugis* shared a common ancestor with *P. maniculatus* after the divergence of *P. keeni* (Fig. 1b). 2) *P. keeni* shared a common ancestor with *P. sejugis* after the divergence of *P. maniculatus* (Fig. 1c). 3) *P. maniculatus* shared a common ancestor with *P. keeni* after the divergence of *P. sejugis* (Fig. 1d). Distributional proximity and morphologic similarity (Hooper and Musser 1964) support the hypothesis that *P. sejugis* was derived from a *P. maniculatus* ancestor native to Baja California, thus seemingly giving support to the first phylogenetic hypothesis (Fig. 1b). However, preliminary mitochondrial DNA (mtDNA, Hogan et al. 1997) and microsatellite (Chirhart et al. 2005) data group *P. sejugis* with *P. keeni* followed by *P. maniculatus* (Fig. 1c). Additionally, these same studies revealed that populations of *P. maniculatus* from Baja California grouped closer to *P. sejugis* than *P. maniculatus* from the central and northern U.S., suggesting the possible existence of an undescribed species in Baja California.

Following the purported late Pliocene origin of the Sea of Cortés, vicariant events that produced the arid islands in this sea likely led to fragmentation of the mainland arid-adapted Pliocene and Pleistocene faunas of Baja California. Correspondingly, these islands are inhabited primarily by desert-adapted mammals with sister taxa on the proximal mainland (Lawlor et al. 2002). Following fragmentation, the island populations are expected to have experienced independent evolutionary trajectories as a result of founder effect, genetic drift and inbreeding. However, mtDNA sequence data suggest that some island populations originated by dispersal followed by restricted gene flow (i. e. isolation by distance) between the island and mainland

populations; such populations would be expected to manifest haplotypic clines across ancestral mainland populations (Hafner et al. 2001).

To test the above hypotheses concerning the origin of insular populations off the coast of Baja California, I considered the relationship between the two species in the *P. maniculatus* species group that are currently recognized in the Baja California Peninsular Desert Region. The larger-bodied *P. sejugis* is restricted to two of the aforementioned arid islands in the Sea of Cortés (Isla San Diego and Isla Santa Cruz), and *P. maniculatus* is apparently distributed across the entire peninsula, though sparsely in Baja Sur. Based on estimates of minimal sequence divergence at the COIII gene (699bp) for *P. sejugis* (n = 6) and one locality of *P. maniculatus* from peninsular Baja California (n = 3), Hafner et al. (2001) suggested that *P. sejugis* will likely prove to be conspecific with *P. maniculatus*. Although *P. sejugis* and *P. maniculatus* are geographically proximal and share allozymic (Avise et al. 1979) and molecular (Hogan et al. 1997, Hafner et al. 2001) similarities that document their close relationship, morphological (Alvarez-Castañeda 2001) and chromosomal (Smith et al. 2000) characteristics support the distinction of these taxa. Further, chromosomal morphological differences provide evidence for a lack of recent gene flow between the two populations of *P. sejugis* that occupy different habitats.

Peromyscus keeni, a coastal species occurring in the deciduous forests and on numerous islands in the Pacific Northwest, diverged from its continental sister species, *P. maniculatus*, in the mid to late Pleistocene (Hibbard 1968). This distributional relationship is thought to be the result of the colonization by the *P. maniculatus* ancestral

stock of Holocene insular zones from an ice-free coastal refugium in mainland British Columbia, Washington, Vancouver Island, or the present-day Hecate Strait following the Wisconsinan (late Pleistocene) glacial retreat (Demboski et al. 1999). However, the identity of the refugium that harbored the ancestor to *P. keeni* is uncertain, as is the associated phylogeographic pattern that resulted in the current coastal distribution of and genetic variation in *P. keeni*.

To assess the microevolution, phylogeography and systematics of the *P. maniculatus* group species along the west coast of North America, a 1439 base pair (bp) fragment of the mitochondrial genome containing the ND3/ND4L/ND4 (NADH dehydrogenase) region of 304 specimens of *P. maniculatus* from Baja California, California, Oregon, and Washington, and for individuals from both insular populations of *P. sejugis* (total n = 20), as well as that of 277 specimens of *P. keeni*, was amplified using the polymerase chain reaction (PCR) and primers listed in Hogan et al. (1997), and subsequently sequenced. This mtDNA region was selected for study because of its utility in previous phylogenetic investigations of closely related species and populations of *Peromyscus* (Hogan et al. 1997) and other vertebrates (Arevalo et al. 1994, Churikov et al. 2001). Analyses of this fragment have produced well-resolved phylogenetic trees for studies of *P. maniculatus*-group species and other groups within this genus (Hogan et al. 1993, 1997; Walpole et al. 1997). Even in comparisons among more divergent species of *Peromyscus*, this mitochondrial fragment has provided strong bootstrap support and Bremer's decay indices for all identified clades (Hogan et al. 1997, Engel et al. 1998). The phylogenetic informativeness of these genes owes to their high nucleotide diversity (Arevalo et al. 1994, Blouin et al. 1998, Churikov et al. 2001) and

subsequent high number of polymorphic sites, which define major haplotype assemblages (Churikov et al. 2001).

Resulting mtDNA sequences were compared within and between these populations and to reference sequences (Hogan et al. 1997) for *P. maniculatus* from the central U.S. in order to identify previously unrecognized partitioning of biodiversity of the deer mice in this region, yield an improved understanding of phylogenetic relationships of these mice, and offer new insights into the post-Pleistocene biogeographic history of Baja California, the westernmost United States and the Pacific Northwest. Finally, patterns revealed by mtDNA variation were tested for geographic concordance with other codistributed taxa of the western coastal region.

CHAPTER II

GENEALOGICAL CONCORDANCE AND THE SPECIFIC STATUS OF *Peromyscus sejugis*

Introduction

Due to the peninsula's dynamic geologic history, heterogeneous habitats and various areas of endemism, the biogeography and evolutionary history of Baja California, Mexico, are complex. Reconstructions of the faunal history of this region are confounded by the debate over vicariance versus dispersal as the explanation for its modern organismal diversity. The current distributions of vertebrates in this region have historically been attributed to late Pleistocene through Holocene dispersal from continental North America (Orr 1960, Savage 1960). More recently, however, the present-day biotic architecture of mainland Baja California has been hypothesized to have arisen via a series of vicariant events during the late Neogene (5.5-1 mya, Murphy 1983; Grismer 1994; Riddle 1995; Riddle et al. 2000a,b,c; Carreño and Helenes 2002).

Following the purported late Neogene origin of the Sea of Cortés, vicariant events that generated the arid islands in this sea likely led to fragmentation of the mainland arid-adapted Pliocene and Pleistocene faunas of Baja California. Correspondingly, these islands are inhabited primarily by desert-adapted mammals with sister taxa on the proximal mainland (Lawlor et al. 2002). Sufficiently isolated island

populations are expected to have experienced independent evolutionary trajectories as a result of founder effect with subsequent genetic drift and inbreeding. However, mtDNA sequence data (Hafner et al. 2001) suggest that some populations of mammals inhabiting islands in the Sea of Cortés originated by dispersal from nonadjacent mainland sources.

Mice of the genus *Peromyscus* are a major component of the mammalian fauna of Baja California and represent one of the most prevalent groups of mammals on the islands in the Sea of Cortés; seven peninsular species of *Peromyscus* are variously present on these islands (Hafner et al. 2001). Two species in the *Peromyscus maniculatus* (Cricetidae, Neotominae) species group are currently recognized in the Baja California Peninsular Desert Region. *Peromyscus maniculatus* is common in Baja California Norte but its occurrence in Baja California Sur is sparse and poorly documented (Hall 1981). *Peromyscus sejugis*, the Santa Cruz Island mouse (Dice 1940, Blair 1950, Hooper 1968, Carleton 1980, Hall 1981), is restricted to two small islands off the coast of Baja California Sur, Isla Santa Cruz (14 km^2) and Isla San Diego (1.3 km^2), in the Sea of Cortés and is considered threatened by the Government of Mexico (Alvarez-Castañeda 2001).

From data for a single individual of *P. sejugis* and one of *P. maniculatus* from Baja California Norte, Hogan et al. (1997) reported a mtDNA (ND3/ND4L/ND4 region) sequence divergence of 2 percent (mistakenly listed as 0.02%). Similarly low sequence divergence at the COIII gene for three individuals of *P. sejugis* from each island and three specimens of *P. maniculatus* from one locality in Baja California Sur led Hafner et al. (2001) to suggest that a more thorough sampling of peninsular *P. maniculatus* would

likely indicate that *P. sejugis* should be included as a subspecies of *P. maniculatus*. The specific distinction of *P. sejugis* and peninsular *P. maniculatus* is based on general morphological characteristics and appears to be supported by karyotypic data. Relative to *P. maniculatus* from peninsular Baja California, Burt's (1932) recognition of *P. sejugis* considered that the latter is larger in size, has a duller pelage, a lighter lateral line, a longer rostrum and noninflated frontals (Alvarez-Castañeda 2001). These taxa also differ for independent pericentric inversions of the plesiomorphic (for *Peromyscus*) acrocentric condition of chromosome 13 (Smith et al. 2000). Character state differences for the presence of distal heterochromatin on the short arm of chromosome 13 suggest a lack of recent gene flow between the two island populations of *P. sejugis* (Smith et al. 2000).

Herein I present an analysis and comparison of sequence variation in the mitochondrial ND3/ND4L/ND4 (NADH dehydrogenase) genes for both populations of *P. sejugis* (n=20) and eight populations of *P. maniculatus* from mainland Baja California (n=96). The objectives of this study were to further assess the taxonomic status of *P. sejugis* and to address the question of whether the populations of *P. sejugis* are the result of either fragmentation of or dispersal from an ancestral mainland population.

Methods

Qiagen purification kits (Qiagen, Inc.) were used to isolate DNA from frozen (-80^{o}C) liver or spleen samples of specimens of *P. sejugis* (Mexico: Baja California del

Sur; Isla San Diego (ISD, n=13), Isla Santa Cruz (ISC, n=7)) and *P. maniculatus* (Mexico: Baja California del Norte: Vallecitos (VLL, n=26); Laguna Hanson, Sierra Juarez (LH, n=29); 16mi S, 5mi E, or 8mi S, 9mi E Valle de Trinidad (VDT, n=31); 3km SW Colonio Vicente Guerrero (CVG, n=3); Mision San Fernando (MSF, n=1); 27 km S Punta Prieta (PP, n=4); Baja California del Sur: 25mi SE Guerrero Negro (GN, n=1); 11 km S Todos Santos (TS, n=1), Fig. 2). The animal use in this research was conducted in accordance with the Guide for Care and Use of Laboratory Animals and was approved by the Texas A&M University Laboratory Animal Care and Use Committee.

Amplification with the polymerase chain reaction (PCR) and sequencing of the 1,439 base pair (bp) fragment of the mitochondrial ND3/ND4L/ND4 genes as well as $tRNA^{Arg}$ and the 3' end of $tRNA^{Gly}$ generally followed the techniques of Arevalo et al. (1994). The primers used for PCR amplification and sequencing included: PI', Marg, ND4L, and Nap2. Amplification reactions (Perkin Elmer/Cetus DNA Thermal Cycler) were conducted with the following reagents and concentrations: 1 μL DNA (approximately 100 ng), 12.3 μL H_2O, 2.5 μL of 10X PCR Buffer II (PE Applied Biosystems), 2.5 μL of 25 mM $MgCl_2$, 0.5 μL BSA, 4 μL of 8 mM dNTPs (Amersham Pharmacia Biotech), 1.0 μL of forward and reverse primers, and 0.2 μL *Taq* (TaKaRa, Japan). Amplifications proceeded in three stages including an initial denaturation cycle at 95°C for five min, followed by 35 cycles of 1 min each at 95°C, 50°C, and 72°C, and concluded with an extension cycle of 10 min at 72°C. Amplified products were purified

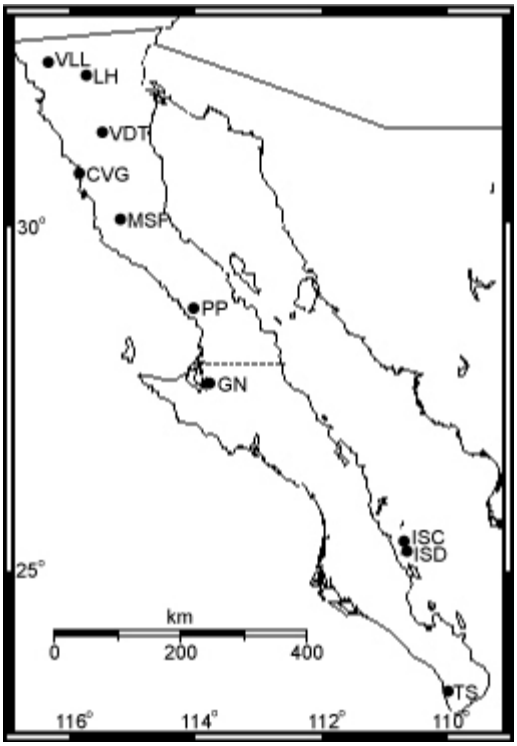


FIG. 2.–Map of Baja California, Mexico, showing locations of populations sampled in this area of study. Designations, specific localities and sample sizes are given in the Methods.

using Exonuclease I in combination with shrimp alkaline phosphatase (ExoSAP, USB).

Each sequencing reaction was performed with a Big Dye sequencing kit (PE Applied Biosystems Inc., Foster City, CA) in a Perkin Elmer/Cetus DNA Thermal Cycler. Amplifications followed the standard Big Dye protocol, and sequences were obtained on an Applied Biosystems 377 automated sequencer. Fragments were sequenced in both directions; sequence alignments and the formation of contigs were conducted using the program Sequencher 4.1.1 (Gene Codes Corporation). Each unique sequence was scored as an individual haplotype. GenBank Accession numbers for each of the haplotypes are presented in the results.

Sequences of haplotypes were compared and their within- and among-locality variation was analyzed. Phenetic and phylogenetic analyses included neighbor-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) approaches in PAUP*4.0b10 (Swofford 2002). MP analyses of the sequence data consisted of heuristic searches using TBR branch swapping with equal weights and 10 random additions. Similar heuristic searches were performed with unequal weights for transitions and transversions (following Hogan et al. 1997). Uncorrected ("p") distances were computed according to Swofford (2002). Modeltest 3.06 (Posada and Crandall 1998) identified HKY+I+G (Hasegawa, Kishino and Yano + invariant sites + gamma distribution for variable sites, Hasegawa et al. 1985) as the most appropriate model of nucleotide evolution for the data. Both NJ and ML analyses employed this model; ML analysis ($\alpha = 0.7648$; $p_{inv} = 0.6163$; transition:transversion ratio = 6.6503) was conducted with heuristic searches using TBR branch swapping with equal weights and

10 random additions. All analyses included reference sequences (Hogan et al. 1997, Chirhart et al. 2001) for single individuals of *P. maniculatus rufinus* (GenBank Accession U40250) and *P. m. austerus* (GenBank Accession U40249); sequence data for *P. melanotis* (Hogan et al. 1997, Chirhart et al. 2001) from Hidalgo, Mexico (GenBank Accession U40247) were used as the outgroup for all analyses. Bootstrap estimates (Felsenstein 1985) based on 1000 replications were obtained for MP and ML analyses.

Haplotypes were also subjected to nested clade analysis (NCA) using TCS (Clement et al. 2000) and GeoDis (Posada et al. 2000) and to subsequent phylogeographic inference. The inference key (Templeton et al. 1995; Appendix I, Templeton 1998) was applied to the GeoDis output in order to attempt to differentiate between alternative biogeographic hypotheses. Although simulation studies (Irwin 2002, Knowles and Maddison 2002) have criticized the NCA inference key's capacity to evaluate alternative phylogeographic hypotheses, the method does provide a statistical framework for characterizing genetically distinct populations *a posteriori* through examination of their geographic distribution and frequency of haplotypes. Templeton (2004) maintained that NCA is complementary to an *a priori* procedure described by Knowles and Maddison (2002).

Results

Each of the island populations of *P. sejugis* exhibited a single unique haplotype: Haplotype 12, ISD (GenBank Accession U40255); Haplotype 13, ISC (GenBank

Accession U40253), differing by a p-distance of 0.7%. The eight population samples of *P. maniculatus* from peninsular Baja California exhibited a total of eleven different haplotypes with a mean p-distance of 0.68%. Haplotype 1 (GenBank Accession DQ077697) was the most frequent and geographically widespread (and thus presumably ancestral) haplotype, characterizing 38 of the 96 individuals of *P. maniculatus* (14 VLL, 12 LH, 10 VDT, 1 CVG, and 1 PP). Other relatively frequent haplotypes included: 17 individuals with Haplotype 2 (6 VLL, 5 LH, and 6 VDT; GenBank Accession DQ077693), 19 with Haplotype 3 (6 VLL, 7 LH, and 6 VDT; GenBank Accession DQ077696), and 8 with Haplotype 4 (5 LH and 3 VDT; GenBank Accession DQ077694). The haplotype exhibited by the single individual from GN (Haplotype 5, GenBank Accession DQ077698) was shared with one individual from CVG. Twelve individuals were characterized by haplotypes that were not observed at any other locality: Haplotypes 6 (GenBank Accession DQ077695) and 7 (GenBank Accession DQ077703; three individuals each from VDT), Haplotype 8 (GenBank Accession DQ077699; 1 CVG), Haplotype 9 (GenBank Accession DQ077702; the single individual from MSF), Haplotype 10 (GenBank Accession DQ077700; 3 PP), and Haplotype 11 (GenBank Accession DQ077701; the single individual from TS).

The mean uncorrected ("p") distance between the sequences of *P. sejugis* and those from the eight populations of *P. maniculatus* was 2%. All analyses (NJ, MP and ML) recovered nearly identical topologies (Fig. 3), with the NJ tree providing more resolution among the haplotypes of *P. maniculatus*. All trees grouped the two populations of *P. sejugis* as sister to the populations from peninsular Baja California and

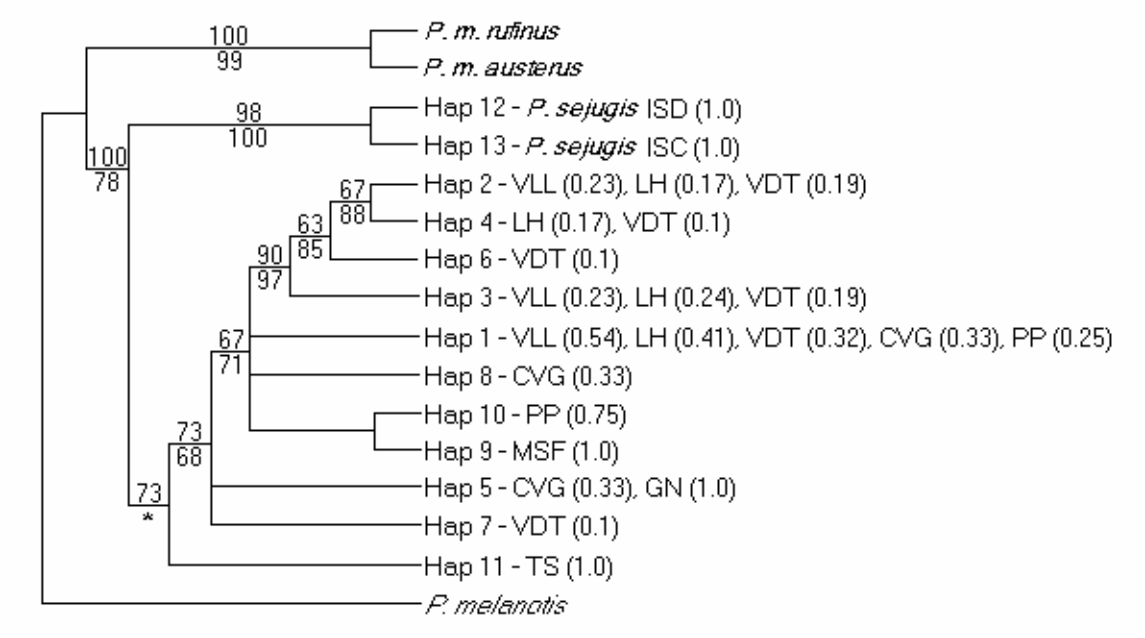


FIG. 3.–Maximum parsimony (MP) tree for ND3/ND4L/ND4 sequence data. Numbers above branches indicate MP bootstrap values, numbers beneath branches indicate corresponding maximum likelihood (ML) bootstrap values, letters represent localities (refer to text, Fig. 1), and numbers in parentheses indicate the frequency of that haplotype in the respective population. Hap = haplotype, * = branch not resolved in ML analysis. Haplotypes 1-11 represent *P. maniculatus*.

these as distinct from and sister to the reference samples of *P. maniculatus austerus* and *P. m. rufinus*. The MP and NJ analyses placed the TS haplotype as basal among the populations from peninsular Baja California; the ML analysis did not resolve the TS haplotype relative to the other peninsular samples (Fig. 3).

The nested clade analysis (Fig. 4) identified phylogroups identical to those in the NJ tree, and the number of intermediate haplotypes identified by TCS in the haplotype network was concordant with the NJ branch lengths (number of steps between phylogroups). The haplotypes (including the presumed ancestral haplotype) from the localities in Baja California Norte and GN (Baja Sur, shared with CVG) clustered with one another and were linked by few missing intermediates (Fig. 4). This phylogroup was associated with the haplotype from southernmost Baja California Sur (TS) and then to the ISD and ISC haplotypes of *P. sejugis*, respectively.

TCS analysis generated no reticulations. GeoDis identified two clades that violated the null hypothesis of panmixia. These included a first-level clade (geographically) between the two populations of *P. sejugis* and a second-level clade (geographically) between the populations of *P. sejugis* and the samples from Baja California. According to the inference key, both significant values led to results that were inconclusive with regard to distinguishing between fragmentation and isolation by distance.

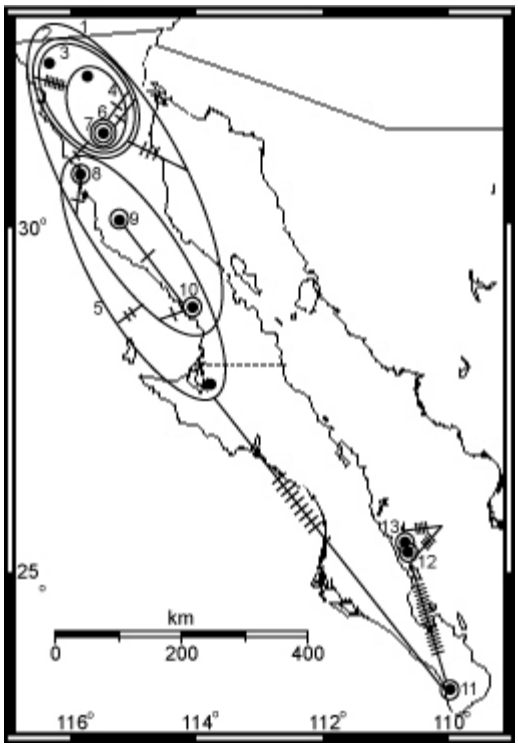


FIG. 4.–Haplotype network superimposed over the geography of Baja California. Ovals represent haplotype distributions, and haplotype numbers (see text, Fig. 2) are to the left of the ovals. Hash marks indicate intermediate (missing) haplotypes.

Discussion

Microevolutionary Factors.– Although our sample sizes of *P. sejugis* were not large, the observation of endemic and invariant haplotypes is supported by previous studies indicating minimal genetic variation in these two small-island populations. For the same individuals as examined in this study, banded karyotypes were invariant within each island population (Smith et al. 2000) and microsatellite variation (Chirhart et al. 2004) was minimal and significantly lower than in reference mainland populations of *P. maniculatus* (Kansas) and the other species in the *P. maniculatus* group. Additionally, Avise et al. (1974) reported only 1.7% per-individual heterozygosity at 23 allozymic loci for a total of 31 individuals of *P. sejugis* representing both islands.

The apparently fixed and endemic haplotypes of the two island populations of *P. sejugis* and the comparatively rich haplotypic diversity of peninsular *P. maniculatus* are consistent with the conclusion (Smith et al. 2000) that there has been a lack of recent gene flow among these populations. The paucity of genetic variation and the pattern of divergence observed for the mitochondrial genes in *P. sejugis* suggest that the origin of these populations has been strongly influenced by genetic drift. Similarly, genetic drift has apparently been the primary process accounting for the differences between the two populations of *P. sejugis*.

Phylogeographic Considerations.–The phenetic, phylogenetic and nested clade analyses of the sequence variation across the ND3/ND4L/ND4 region of the mtDNA (Figs. 3 & 4) are entirely consistent in indicating an overall genetic continuity among the

populations of *P. maniculatus*, and in clustering the two island populations of *P. sejugis* outside of the group of mainland populations and distinct from one another. Phylogeographic analysis (Fig. 4) did not provide definitive distinction between past fragmentation and isolation by distance as the mechanism responsible for the current pattern of mtDNA variation between *P. sejugis* and the *P. maniculatus* from Baja California. However, I favor the fragmentation model for the following reasons: (1) The autapomorphies mentioned above establish the mainland and island populations as distinct evolutionary units with no evidence of the homogenizing effects of gene flow. (2) Islands in this same archipelago (Isla Espiritu Santo and Isla San Jose), and thus presumably Isla San Diego and Isla Santa Cruz, were severed from the present-day La Paz Peninsula during a Pleistocene glacial advance 25,000-17,000 years ago (Beal 1948), thus potentially fragmenting a formerly contiguous ancestral population. (3) The geologic transgressions that resulted in the present sea level 6,000 years ago (Avise et al. 1974) would likely have precluded recent waif dispersal and gene flow. Although a more intensive sampling of southern peninsular deer mice might reveal intermediate sequences that would support an isolation by distance-based origin of *P. sejugis*, the scarcity of *P. maniculatus* in Baja California Sur will likely preclude obtaining the samples necessary to test this hypothesis.

Taxonomy.– Due to the complications of insular allopatry, any decision pertaining to the specific distinction of *P. sejugis* is necessarily subjective. Given the minimal distribution and threatened status of *P. sejugis*, however, the specific recognition of *P. sejugis* has important conservation implications and should be carefully

considered. Although our analysis of sequence variation across the ND3/ND4L/ND4 region confirms initial observations (Hogan et al. 1997, Hafner et al. 2001) of a low level of mtDNA divergence between *P. sejugis* and the *P. maniculatus* from Baja California, this alone does not warrant the conclusion that *P. sejugis* should be considered as conspecific with *P. maniculatus*. Moreover, the pattern of genealogic concordance of the morphologic (Burt 1932), chromosomal (Smith et al. 2000), microsatellite (Chirhart et al. 2004) and mtDNA variation supports the conclusion that *P. sejugis* and the *P. maniculatus* from Baja California are independent evolutionary lineages and represent separate phylogenetic species (Cracraft 1983, Nixon and Wheeler 1990) as modified by inferences from coalescent theory (*sensu* Avise and Ball 1990, Avise and Wollenberg 1997).

A similar, although less compelling, argument can be applied to the hypothesis that the two island populations of *P. sejugis* should be recognized as separate phylogenetic species. Although mtDNA, karyotypic, microsatellite and morphologic data are concordant in indicating a lack of recent gene flow between the two island populations of *P. sejugis*, the differences in these characteristics reflect less time since divergence than do those that distinguish the two populations of *P. sejugis* from peninsular *P. maniculatus*. Whereas the difference between the alternate ND3/ND4L/ND4 haplotypes of the two populations of *P. sejugis* was 0.7%, the mean sequence divergence between the haplotypes of *P. sejugis* and those of peninsular *P. maniculatus* was 2.0%. The NCA primary-level nesting of the populations of *P. sejugis* and secondary-level nesting for *P. sejugis* versus the peninsular deer mice is consistent

with the hypothesis that there has been less time since the divergence of the two island populations of *P. sejugis*. Karyotypically, the *P. sejugis* from Isla Santa Cruz differ from those on Isla San Diego by the presence of distal heterochromatin on the short arm of chromosome 13, whereas both island populations of *P. sejugis* differ from peninsular *P. maniculatus* by a unique inversion of this same chromosome (Smith et al. 2000). Although the two island populations of *P. sejugis* were either monomorphic for the same allele or shared the same alleles at similar frequencies at eight microsatellite loci, these populations exhibited a fixed difference at one microsatellite locus and unique low frequency alleles at two other loci (Chirhart et al. 2004). Alvarez-Castañeda (2001) reported minor qualitative differences in the shape of the nasals and angle of the sutures between the frontals and parietals between specimens of *P. sejugis* from the two islands but noted that external and cranial measurements did not differ. Given the circumstances and available data, I conclude that *P. sejugis* should retain its specific distinction and that the two island populations of *P. sejugis* at least constitute evolutionarily significant units (for review see Hey et al. 2003 and references therein), which, for conservation purposes, should be considered as separate entities and managed independently.

CHAPTER III

PHYLOGENETIC IMPLICATIONS OF MITOCHONDRIAL-DNA SEQUENCE VARIATION IN DEER MICE FROM CALIFORNIA

Introduction

The monophyly of the *P. maniculatus* group and the systematic relationships of its earliest-diverged taxa (*P. maniculatus*, *P. polionotus*, and *P. melanotis*) have been accepted based on morphologic, cytogenetic and molecular data (reviewed by Chirhart et al. 2005). Within this group, however, the phylogenetic relationships and evolutionary history of two peripherally distributed species, *P. sejugis* (endemic to Isla San Diego and Isla Santa Cruz in the Gulf of California) and *P. keeni* (coastal deciduous forests from northern Washington to southeastern Alaska and southwestern Yukon), remain problematic. Allozymic (Avise et al. 1979) and cytogenetic data (Gunn and Greenbaum 1986, Smith et al. 2000) associate *P. keeni* and *P. sejugis* with *P. maniculatus,* but the divergence of these species was apparently too recent for the evolution of synapomorphies in these characters, yielding an unresolved trichotomy that could be explained by three alternative phylogenetic hypotheses (Fig. 1). Preliminary data comparing sequence variation at the ND3/ND4L/ND4 region of the mitochondrial genome from *P. sejugis* and *P. keeni* (Hogan et al. 1997) suggest the geographically improbable sister-group relationship between these species, and the hypothesis that *P.*

keeni shared a common ancestor with *P. sejugis* after the divergence of *P. maniculatus* (as opposed to a sister-group relationship between *P. keeni* and *P. maniculatus* or *P. sejugis* and *P. maniculatus*). However, the critical sample sizes and data relative to testing these alternative hypotheses are lacking.

Further confounding this issue, distributional proximity, morphologic similarity (Hooper and Musser 1964) and molecular data (see Chapter II) clearly support the hypothesis that *P. sejugis* was derived from a *P. maniculatus* ancestor native to Baja California. Comparisons of mtDNA sequences associate the *P. maniculatus* from Baja California (*P. m. coolidgei* and *P. m. gambelii*) with *P. sejugis* (Fig. 1) and nest this *P. sejugis*-like haplogroup between *P. sejugis* and *P. keeni* rather than clustering it with the *P. maniculatus* from the central (Colorado) and northern (Washington) U.S. (Hogan et al. 1997). These data suggest that *P. maniculatus sensu stricto* is polyphyletic (i.e. circumscribes more than one evolutionarily distinct species). Based on size, tail length, coloration, and dimensions of the brain case, rostrum and incisive foramina (Hooper 1944), the current taxonomy of *P. maniculatus* recognizes 4 subspecies in the Baja California/California (CA) region. *Peromyscus m. coolidgei* is restricted to the Baja California peninsula, whereas *P. m. sonoriensis* and *P. m. gambelii* occur in the northern peninsula and extensively through southern CA and northward (Fig. 5). *Peromyscus m. rubidus* is strictly coastal and ranges from the northern boundary of the San Francisco Bay (SFB) in CA to the Oregon/Washington border (Fig. 5). As these designations reflect morphotypes, of particular interest is whether such phenotypic variation is corroborated by comparably partitioned genetic variation. Lansman et al. (1983)

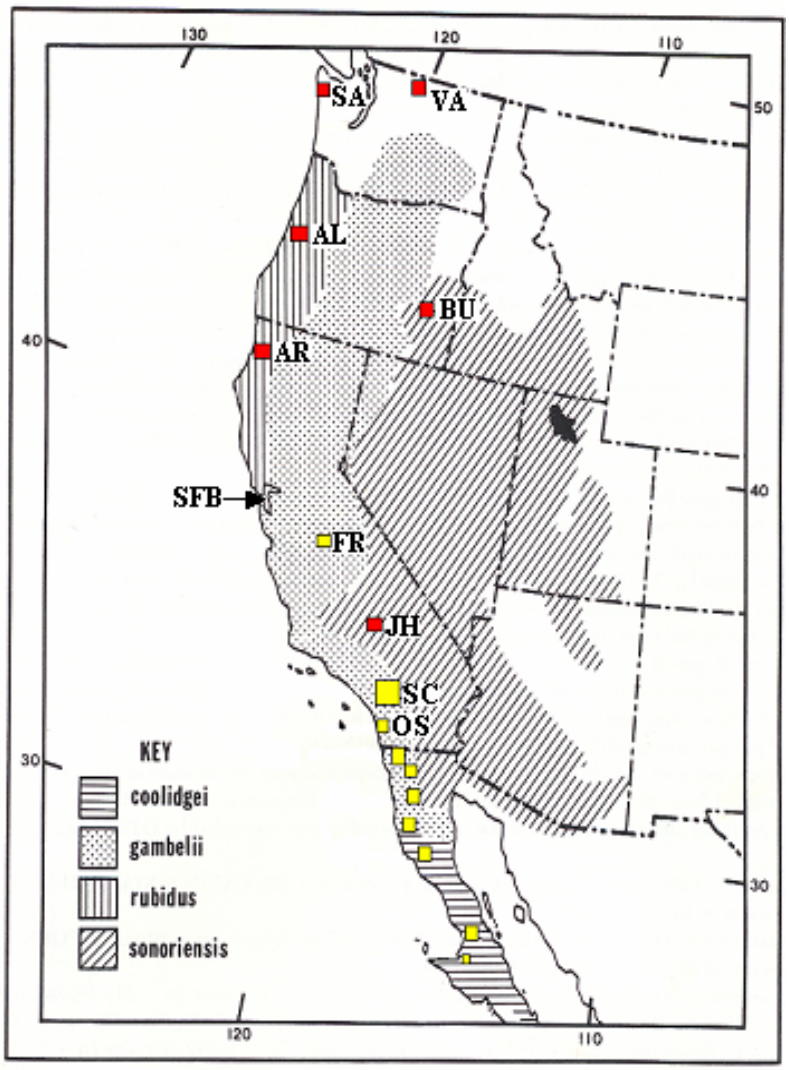


FIG. 5.–Collecting localities for populations of *P. maniculatus* from California, Oregon and Washington. Box sizes reflect sample sizes. Subspecific distinctions are those of Hall (1981). OS = Oceanside, SC = South California (Boulder Bay (BB), Big Bear (BBR), Heart Bar (HB)), JH = Johannesburg, FR = Fresno, AR = Arcata, BU = Burns, AL = Alsea, VA = Varden Creek, SA = Satsop, SFB = San Francisco Bay. For localities in Baja California, see Chapter II.

observed restriction fragment length polymorphism (RFLP) in the mtDNA of *P. maniculatus* that, based on limited collecting localities in California, roughly approximated the above subspecific distinctions. However, RFLPs underestimate sequence divergence, thus leaving informative characters undetected, and are therefore not as phylogenetically or taxonomically informative as are sequence data. An exhaustive sequence-based consideration of intraspecific taxonomy in this western coastal region should confirm whether subspecific distinctions are genetically supported and elucidate whether they reflect the hypothetical polyphyly among western-distributed *P. maniculatus*.

The purpose of this analysis was to identify the approximate geographic boundary of the *P. sejugis*-like haplogroup, determine the relationship of this haplogroup to central and northern *P. maniculatus* and to *P. keeni* and assess the intraspecific taxonomy of *P. maniculatus* along the west coast of North America. For this analysis, sequences of the ND3/ND4L/ND4 region of the mitochondrial genome were obtained for 99 *P. maniculatus* from southern, central and northern California (Fig. 5) and 109 *P. maniculatus* from Oregon and Washington. These results were compared to reference sequences for *P. keeni*, *P. sejugis* and *P. maniculatus* from Colorado. These analyses support the hypothesis of a currently unrecognized partitioning of the biodiversity of deer mice in this region, yield an improved understanding of phylogenetic relationships of these mice, and offer new insights into the post-Pleistocene biogeographic history of Baja California and the western-most United States.

Methods

Frozen tissues (stored at –80°C) of *P. maniculatus* (California: Oceanside (n=22), Boulder Bay (n=3), Big Bear (n=10), Heart Bar (n=26), Fresno (n=22), Johannesburg (n=5), Arcata (n=11); Oregon: 28mi S, 6mi E Burns (n=20), 9mi E Alsea (n=19); Washington: Gray's Harbor (n=43), Varden Creek (n=27)) were available from prior materials collected under TAMU Animal Use Protocol #92-1054 and in association with prior research conducted under NIH Grant GM27014-12/16, "Chromosomal rearrangement incorporation in mammals" to Ira F. Greenbaum, Department of Biology, Texas A&M University. Reference sequences for *P. sejugis* and *P. keeni* were obtained from results reported in other chapters of this dissertation. The use of tissues from these specimens was in accordance with the Guide for Care and Use of Laboratory Animals (U.S. Department of Health and Human Services as approved by the Texas A&M University Laboratory Animal Care Committee AUP #2000-275).

Techniques for DNA isolation, amplification and obtaining sequences were as described in Chapter II. The haplotype sequences for samples of *P. maniculatus* from California, Oregon and Washington were compared to sequences obtained for the same region of the mitochondrial genome for reference samples of *P. keeni, P. sejugis* and *P. maniculatus*. The reference sequences were the most common haplotype from each of the following localities: *P. keeni*: U.S.A.; Washington, Okanogan Co, Lone Fir Campground and Gray's Harbor Co, 3.0 mi N, 1.0 mi E Grisdale, Satsop Workcamp; *P. m. austerus*: Canada, British Columbia; Vancouver Island, 35.7 km W Port Alberni,

Sproat Lake (n=1); *P. m. rufinus*: U.S.A., Colorado; 7.2 km N, 8.8 km W Central City, Elk Park (n=1); *P. sejugis*: Mexico; Baja California del Sur, Isla San Diego (n=13), Isla Santa Cruz (n=7)) and *P. maniculatus* (Baja): Mexico: Baja California del Norte: Vallecitos, Sierra de San Pedro Martir (n=20); Laguna Hanson, Sierra Juarez (n=28); 16mi S, 5mi E or 8mi S, 9mi E Valle de Trinidad (n=28); 3km SW Colonio Vicente Guerrero (n=3); 25mi SE Guerrero Negro (n=1)).

Phenetic and phylogenetic analyses followed those described in Chapter II and included neighbor-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) approaches in PAUP* version 4.0b10 (Swofford 2002). Modeltest 3.06 (Posada and Crandall 1998) identified HKY+I+G (Hasegawa, Kishino and Yano + invariant sites + gamma distribution for variable sites, Hasegawa et al. 1985) as the most appropriate model of nucleotide evolution for the data. Both NJ and ML analyses employed this model; ML analysis ($\alpha = 0.7618$; $p_{inv} = 0.4641$; transition:transversion ratio = 4.4181) was conducted as in Chapter II. Sequence data for *P. melanotis* (Hogan et al. 1997, Chirhart et al. 2001) from Hidalgo, Mexico (GenBank Accession U40247) were used as the outgroup for all analyses. Bootstrap estimates (Felsenstein 1985) based on 1000 replications were obtained for NJ and MP analyses.

Results

The percent sequence divergences within and between the observed haplotypes were obtained using a pairwise distance matrix in PAUP* and are summarized in Table

1. Haplotype numbers and their inclusive localities are shown in Table 2. Analysis of the ND3/ND4L/ND4 region of the mtDNA revealed moderately low percent sequence divergence between *P. sejugis* and *P. maniculatus* from Baja California (2.08). The pairwise distance between central/northern (Colorado, Oregon and Washington) *P. maniculatus* and *P. keeni* (4.40%) was comparable to that of *P. keeni* versus *P. maniculatus* from Baja California (3.52%) and *P. sejugis* (3.70%). The central/northern *P. maniculatus* and *P. maniculatus* from Baja California exhibited a high sequence divergence at 4.48%. Sequence divergences for central/northern *P. maniculatus* compared to S. California (4.17%) and Fresno (3.99%) were likewise high. These values are similar to pairwise distances between *P. maniculatus* from Baja California and the populations from Johannesburg (3.92%) and Arcata (3.76%). Alternatively, percent sequence divergences of central/northern *P. maniculatus* versus Johannesburg (0.80) and Arcata (1.75) were low, as were those between *P. maniculatus* from Baja California and the S. California (0.80) and Fresno (0.85) populations.

The NJ analysis using the HKY model of nucleotide evolution produced an optimal tree (Fig. 6) that identified all but one of the same deep clades as the strict consensus MP tree and the ML tree. The NJ and MP bootstrap and ML trees (Figs. 7 & 8) placed Haplotype 11 (TS) as sister to (rather than included in) the *P. sejugis* clade. All analyses of the sequence data grouped the haplotypes of *P. maniculatus* from Baja/S. California/Fresno and placed them in a cluster that otherwise comprised the two populations of *P. sejugis*. This clade clustered to the reference samples of *P. keeni*

Table 1
Mean percent sequence divergence among haplotypes for the ND3/ND4L/ND4 region of the mtDNA for the deer mice (*Peromyscus*) from California and for each reference sample examined. C/N indicates individuals from the central and northern United States. Measures are based on "p" distances.

Taxon or Locality	1	2	3	4	5	6	7	8
1 *P. manic* (C/N)	-	4.40	4.73	4.48	4.17	3.99	0.80	1.75
2 *P. keeni*		-	3.70	3.52	3.13	3.02	4.10	4.23
3 *P. sejugis*			-	2.08	1.80	1.75	4.11	4.22
4 *P. manic* (Baja)				-	0.80	0.85	3.92	3.76
5 S California					-	0.46	3.75	3.58
6 Fresno						-	3.51	3.33
7 Johannesburg							-	0.90
8 Arcata								-

Table 2
Haplotypes of Pacific coastal deer mice and the localities at which they are found. Haplotypes 1 through 13 correspond with those in Chapter II. Locality abbreviations are consistent with those in Figs. 2 and 5. Numbers preceding localities indicate the number of individuals from that locality exhibiting the respective haplotype (Hap). C/N = central/northern.

Hap #	Baja/S California Locality	Hap #	C/N Locality
1	1 PP, 1 CVG, 10 VDT, 12 LH, 14 VLL, 7 OS	41	2 AR, 19 VA
2	6 VDT, 5 LH, 6 VLL	42	4 JH
3	6 VDT, 7 LH, 6 VLL	43	1 JH
4	3 VDT, 5 LH	44	5 AR
5	1 GN, 1 CVG, 2 HB, 1 FR	45	2 AR
6	3 VDT	46	2 AR
7	3 VDT, 1 BB, 1 HB, 3 FR	47	1 VA
8	1 CVG	48	1 VA
9	1 MSF	49	1 VA
10	3 PP	50	1 VA
11	1 TS	51	1 VA
12	13 ISD		
13	7 ISC		
14	4HB, 3 FR		
15	1 BBR, 2 HB, 3 FR		
16	1 BBR, 1 HB, 2 FR		
17	1 HB, 3FR		
18	1 HB, 2 FR		
19	1 BBR, 2 FR		
20	1 HB, 1 FR		
21	1 BBR, 1 FR		
22	1 BBR, 1 FR		
23	3 OS		
24	3 OS		
25	2 OS		
26	2 OS		
27	1 OS		
28	1 OS		
29	1 OS		
30	1 OS		
31	1 OS		
32	1 HB		
33	1 HB		
34	1 HB		
35	1 HB		
36	1 HB		
37	1 BB		
38	1 BB		
39	1 BBR		
40	1 BBR		

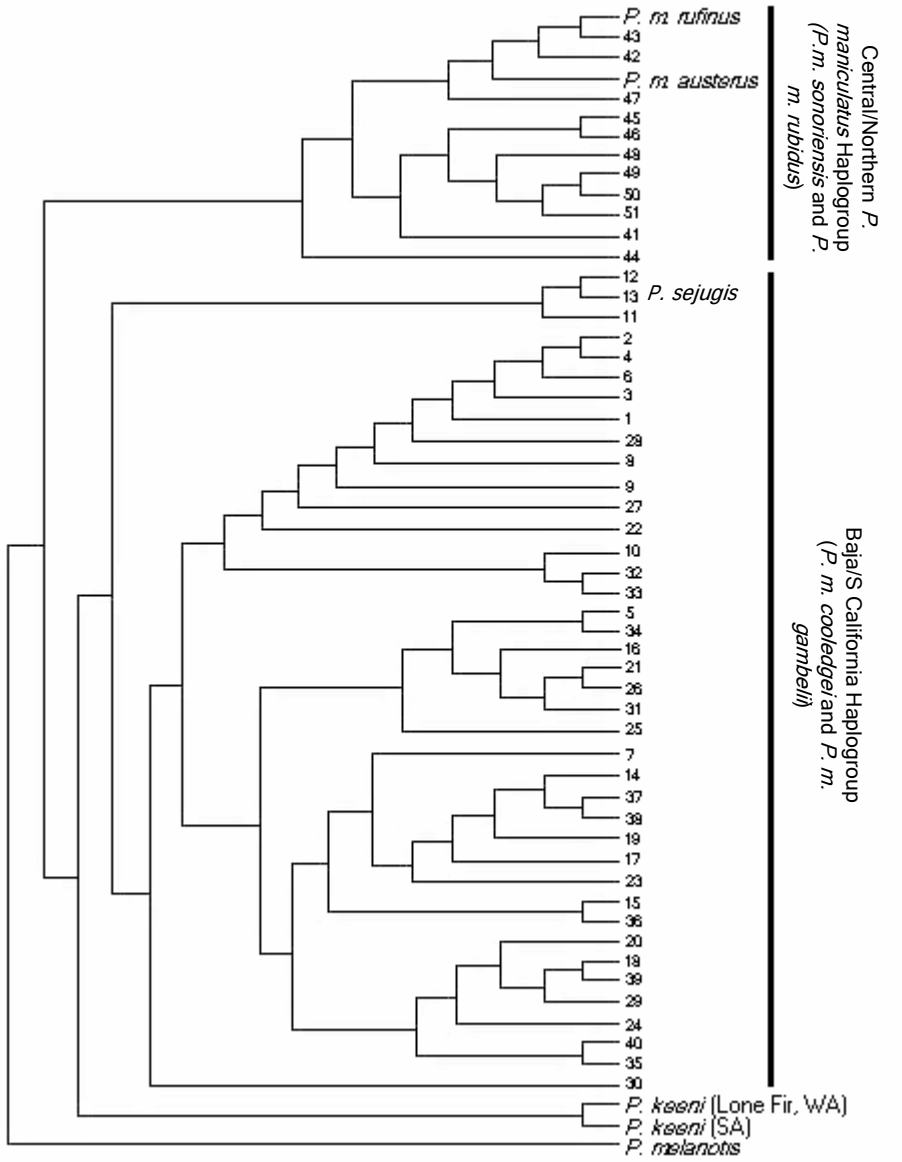


FIG. 6.–Neighbor-joining tree using HKY model of nucleotide evolution. Numbers = haplotypes (See Table 2).

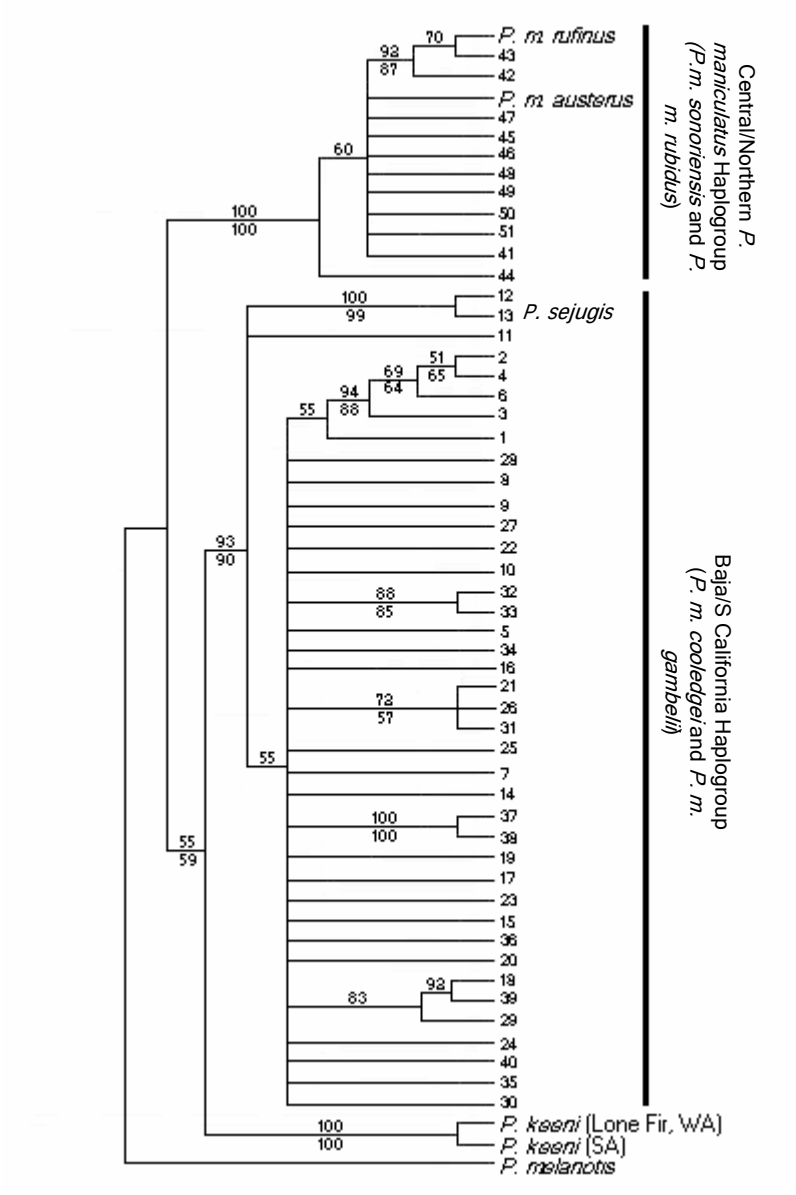


FIG. 7.–Maximum parsimony strict consensus tree. Numbers at branch tips = haplotypes (See Table 2). Numbers above lines = NJ (HKY) bootstrap values. Numbers below lines = MP bootstrap values (both based on 1000 replications).

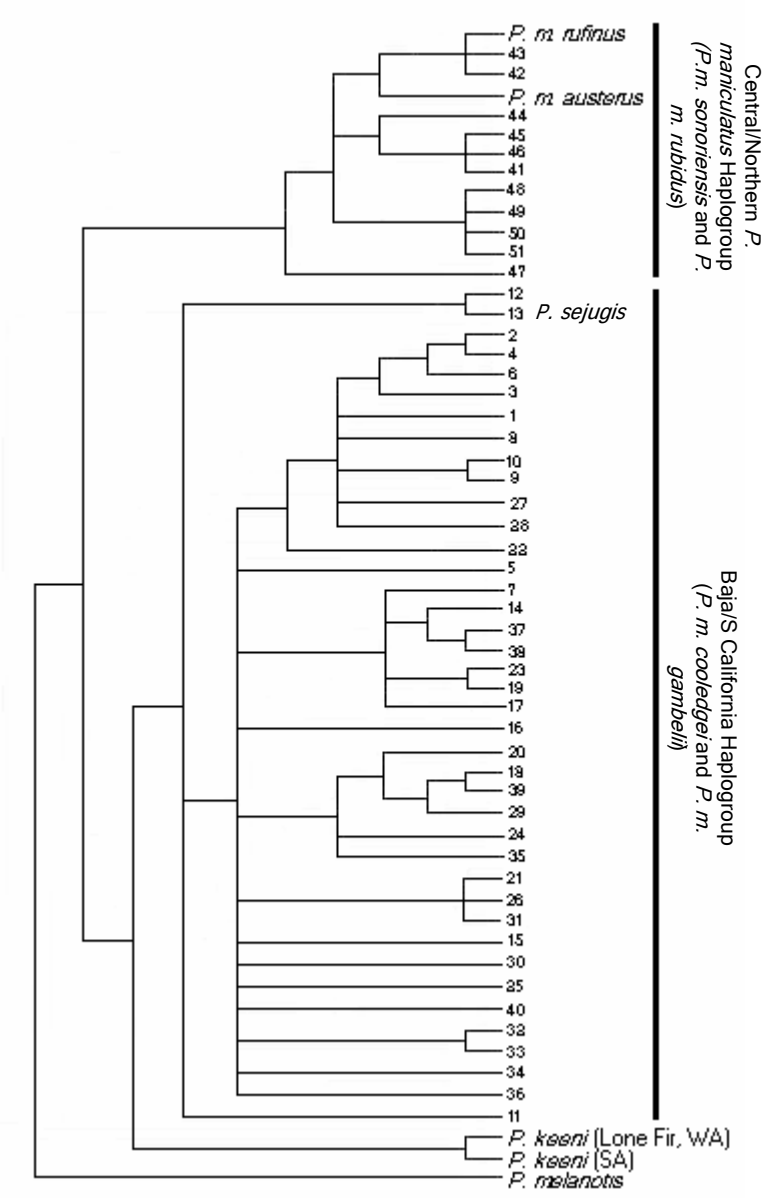


FIG. 8.–Maximum likelihood consensus tree. Numbers at branch tips = haplotypes (See Table 2).

before clustering to the central/northern *P. maniculatus* (including the OR and WA samples). No populations were reciprocally monophyletic (i.e. all shared haplotypes with other populations).

Discussion

The genetic distances (Chapter II and Table 1) and gene trees (Figs. 1, 6, 7 & 8) clearly associate the populations of *P. sejugis* with those of the deer mice from mainland Baja California. The sequence divergence among the haplotypes of *P. maniculatus* from mainland Baja California averaged 0.44%, and that between these and the haplotypes of *P. sejugis* averaged 2.08%. These differences are more consistent with those obtained for geographic conspecific populations than with sequence divergences generally characteristic of even closely related species (Bradley and Baker 2001) and are consistent with the aforementioned evidence for the origin of *P. sejugis* from *P. maniculatus* occurring in Baja California. The close phylogenetic association of *P. sejugis* and the deer mice from mainland Baja California is further supported by the phylogenetic analyses (Figs. 6, 7 & 8). However, as discussed in Chapter II, the complications of insular allopatry, the threatened status of *P. sejugis* and the pattern of genealogic concordance among genetic and morphologic variation support the conclusion that *P. sejugis* should retain its specific distinction relative to the *P. maniculatus* from Baja California. Given this conclusion, the following discussion recognizes the deer mice from peninsular Baja and Southern California as constituting a

haplogroup within, but distinct from, the aforementioned *sejugis*-like haplogroup. Sequence divergence between the central/northern populations of *P. maniculatus* (Colorado, Oregon and Washington) and the *P. maniculatus* from Baja California was 4.48%, whereas that between central/northern *P. maniculatus* and the biologically distinct species *P. keeni* was 4.40%. The sequence divergence between the two clades of *P. maniculatus* is therefore comparable to that seen between two well-established species in the *P. maniculatus* species group and supports the polyphyly of *P. maniculatus*. Because the haplotypes of deer mice from the Southern California (Oceanside, Boulder Bay, Big Bear, Heart Bar) and Fresno populations align with those of *P. maniculatus* from Baja (and *P. sejugis*), and the haplotypes of individuals from Arcata cluster with those from *P. maniculatus* from the central and northern U.S., I conclude that the northern geographic boundary between these clades lies in the region between Fresno and Arcata, CA (Fig. 5 - light boxes indicate populations with Baja/S California haplotypes; dark boxes indicate populations with central/northern *P. maniculatus* haplotypes). The lack of reciprocal monophyly for any population suggests that there has been recent gene flow among the populations within each of these clades, though this could be attributed to incomplete lineage sorting.

That the Johannesburg population clusters with the central/northern populations of *P. maniculatus* indicates an eastern boundary to the Baja/S California haplogroup that is probably coincident with the Sierra Nevada Mountains (spanning from ~35^{o} to 39^{o} N latitude). This suggests a lack of gene flow between populations east and west of this mountain range. Thus, the present data suggest that the populations of deer mice

occurring west of the Sierra Nevadas and south of the San Francisco Bay area comprise the Baja/S California haplogroup. Conversely, as indicated in Table 1 and the phylogenetic trees (Figs. 6, 7 & 8), populations east of the Sierra Nevada Mountains likely exhibit central/northern *P. maniculatus*-like haplotypes.

As there is no fossil evidence to document that any of the rodent species of western North America occurred in this region prior to the Pleistocene (~12,000 ybp), it is presumed that present-day species became established in Pleistocene or Post-Pleistocene times. The vicariant and geomorphic events that produced the current topographic features of west-central California occurred during this epoch (Hooper 1944). One of these features, the Sonoma-Marin gap just north of San Francisco Bay (Fig. 5) provides a break in the present-day faunal continuity along the humid coastal belt of California. This gap, characterized by an abrupt transition in climate, altitude and habitat, marks the division of coastal California into the Northern humid coast and the Southern humid coast faunal subregions (Hooper 1944), and is thus flanked by independent populations or species. The faunal distributions of the California interior are also broken at approximately this same latitude by the Sacramento-San Joaquin Delta, which further precludes gene flow between populations to its north and south (Matocq 2002a). Historic separation of the northern and southern faunas of the interior of California was due ostensibly to the geographic presence of the San Francisco Bay and to the Pleistocene glaciation of its river (Sacramento and San Joaquin) drainages (Matocq 2002b).

Many of the genera and species of rodents in California exhibit discontinuous distributions corresponding to the above physiographic barriers. These distributions are manifest in distinct species or subspecies north and south of the San Francisco Bay/ Sacramento-San Joaquin Delta area (Hooper 1944). These include: *Spermophilus beecheyi beecheyi* (S coastal and interior) and S. *b. douglasii* (Beechey ground squirrels, N coastal and exterior); *Tamias merriami pricei* (Merriam chipmunk, S coastal), *T. sonomae alleni* (N coastal) and *T. s. sonomae* (Sonoma chipmunks, N interior); *Sciurus griseus nigripes* (S coastal and interior) and *Sciurus griseus griseus* (N coastal and interior, Western gray squirrels); *Thomomys bottae bottae* (S coastal), *T. b. diaboli* (S interior), *T. b. minor* (N coastal) and *T. b. agricolaris* (Botta pocket gophers, N interior); *Dipodomys heermanni berkeleyensis*, *D. h. goldmani*, *D. h. heermanni* (all S interior) and *D. h. californicus* (Heermann kangaroo rats, N coastal and interior); *Microtus californicus californicus* (S coastal and interior), *M. c. paludicola* (S interior), *M. c. eximius* (N coastal and interior) and *M. c. aestuarinus* (red-backed voles, N interior); and *Neotoma fuscipes annectens* (S coastal and interior), *N. f. perplexa* (S interior), *N. f. monochroura* (N coastal) and *N. f. fuscipes* (dusky-footed wood rats, N interior, Matocq 2002b). Other vertebrate taxa whose distributional and evolutionary histories have been influenced by the San Francisco Bay and Sacramento-San Joaquin Delta include: *Ensatina eschscholtzi* (ensatina, salamander, Wake 1997), *Contia tenuis* (sharp-tailed snake, Feldman and Spicer 2002), *Lampropeltis zonata* (California mountain kingsnake, Rodriguez-Robles et al. 1999), *Strix occidentalis* (Spotted owl, Gutiérrez and Barrowclough 2005), *Sorex ornatus* (ornate shrew, Maldonado et al. 2001) and *Puma*

concolor (mountain lion, Ernest et al. 2003). *Ensatina* and *Contia* each exhibit a northern pocket of individuals that shares variation with the southern clade, yet this shared variation is thought to be the consequence of retained ancestral polymorphism (i.e. lineage sorting) rather than gene flow across the San Francisco Bay area (Feldman and Spicer 2002). The southern (S coastal and interior, i.e. southern CA and Fresno) and northern (N coastal and interior, i.e. Arcata) populations of *P. maniculatus* studied herein appear to conform to the distributional pattern marked by a genetic division at the San Francisco Bay/Sacramento-San Joaquin Delta area and exhibit phylogenetic propinquity with populations to their south or north, respectively.

The assertion that the geographic boundary between the clades of *P. maniculatus* lies in the region between Fresno and Arcata, and more specifically in the San Francisco Bay area, supports RFLP data from mtDNA (Lansman et al. 1983) that indicate separation of populations of *P. maniculatus* into two distinct clades in California. The Southern California clade (samples from Tulare (just south of Fresno), Alameda, Amador, Riverside, Mono and Inyo, CA) extends northward to the San Francisco Bay/ Sacramento-San Joaquin Delta boundary and is "distantly related to" the Central States clade (samples from Humboldt Co., CA (in which Arcata is located), OR, WA, CO, AZ, NM and central states from TX to Canada), differing from it by at least 8 mutational steps. Morphologic data (Hooper 1944) distinguishing *P. m. gambelii* (S of the boundary) and *P. m. rubidus* (N coastal, Fig. 5) also correspond to this geographic division. The interspecific-level sequence variation between the haplogroups of western deer mice, as well as the genealogical concordance afforded by RFLP (Lansman et al.

1983) and morphologic (Hooper 1944) data, and the geographic concordance with co-distributed taxa, support the hypothesis that the populations south of this boundary, including those in Baja California, characterize an independent evolutionary unit and thus represent a cryptic species nested within *P. maniculatus*.

The recognition of a sibling species of *P. maniculatus* south of the San Francisco Bay/Sacramento-San Joaquin Delta area and west of the Sierra Nevada Mountains generally corresponds with the subspecific taxonomy in this region. The populations of *P. m. rubidus* and *P. m. sonoriensis* (Fig. 5) align with the central/northern *P. maniculatus* (a group that corroborates the Central States assemblage of Lansman et al. (1983)), whereas the sampled populations of *P. m. gambelii* (representing the Southern California assemblage of Lansman et al. 1983) are phylogenetically distinct and align with those of *P. m. coolidgei* from Baja California.

Though the data herein implicate the above physiographic barriers in precluding gene flow between clades of *P. maniculatus* in western North America, many questions concerning the distributional limits of the inclusive groups of mice remain unresolved. As the interior distribution of *P. m. gambelii* north of the putative boundary was not sampled in this study, it is not clear to which clade these populations are associated. And because the mountain ranges of northern and central California diminish in extreme southeastern California, it is not known if the Baja/S California haplogroup extends eastward from southern California and northern Baja California into southwestern Arizona (within the range of *P. m. sonoriensis*, Fig. 5). Further, the precise location of the northern geographic boundary of the Baja/S California haplogroup remains

unidentified. To finely resolve these distributional issues will require further sampling and analyses in north- and southeastern California as well as in the region between Fresno and Arcata.

The ND3/ND4L/ND4 sequence data were also used to assess the genealogical relationships of *P. sejugis* and the Baja/S California haplogroup relative to the northwestern peripheral species *P. keeni*. The mean sequence divergence between the Baja/S California haplogroup and *P. keeni* was 3.52%, and these nest as sister groups before clustering to the central and northern populations of *P. maniculatus* (Figs. 6, 7 & 8). As previously suggested (Hogan et al. 1997), these data support the seemingly geographically improbable sister-group relationship between *P. sejugis*, the deer mice from Baja and southern California and *P. keeni*. Both distance and parsimony analyses therefore confirm the close genetic association and phylogenetic propinquity among *P. keeni*, the purported cryptic species from Baja/S California and *P. sejugis* and support the hypothesis that this group of species shared a common ancestor after their divergence from a *P. maniculatus* ancestral stock.

CHAPTER IV

PHYLOGEOGRAPHY OF THE NORTHWESTERN DEER MOUSE: IMPLICATIONS FOR A PLEISTOCENE REFUGIUM

Introduction

The standard island biogeographic model of island colonization is ostensibly implied by the floral and faunal biogeography in the Pacific Northwest (PNW) of North America, which is generally characterized by the presence of "coastal" species whose sister taxon is a more widely distributed "mainland" species. This distributional relationship is thought to be the result of the founding of Holocene insular zone populations by individuals from an ice-free refugium source in either mainland British Columbia or Washington following the Wisconsinan (late Pleistocene) glacial retreat (Demboski et al. 1999). However, two coastal refugia and thirteen nunataks (mountain refugia) flanked the Cordilleran ice sheet of the PNW ~13,000 ybp, including one on Vancouver Island and one on the Queen Charlotte Islands (Graham and Moresby Islands; Pielou 1991).

It is thought that one or both of these refugia, and/or a reputed refugium in the present-day Hecate Strait (between the Queen Charlotte Islands and mainland British Columbia) served as a sanctuary for many Pleistocene organisms during the height of the Pleistocene glacial advance (Orr and Orr 1996). Patterns of genetic variation in

similarly-distributed vertebrate species (e.g. sticklebacks, O'Reilly et al. 1993; song sparrows, Zink and Ditmann 1993; and black bears, Byun et al. 1997) implicate the Queen Charlotte Islands as a central Pleistocene glacial refugium (the Haida Gwaii refugium), though isolation of these species in other purported refugia seems equally likely (Orti et al. 1994, Demboski et al.1998).

Climatically-induced habitat fluctuations and potent geological events (e.g. cycles of marine transgression/regression and glaciation/postglacial flooding, tectonic shifts, orogenies) of the Pleistocene and Holocene promoted cycles of isolation and secondary contact among populations in the Pacific Coastal region of North America. Evidence of expansions in population size and geographic range can be expected to reflect 1) historical geographic changes, 2) climatic changes and/or 3) the regression of physiographic barriers. Testing hypotheses pertaining to the relationship between the geographic history and evolution of a species (intraspecific phylogeography) can reveal the mechanisms of such expansions and dispersals and allow the formulation of hypotheses concerning vicariant events.

Perhaps no species of the Pacific Northwest is better suited to a phylogeographic test of the mainland refugium hypothesis than is the northwestern deer mouse, *Peromyscus keeni*. This terrestrial species occurs widely in the PNW (Greenbaum 1999) in the coastal deciduous forests of the coastal mainland, numerous coastal islands and various oceanic islands including the Queen Charlotte Islands (Fig. 9). *Peromyscus keeni* is well documented to have diverged from its mainland sister species, *P.*

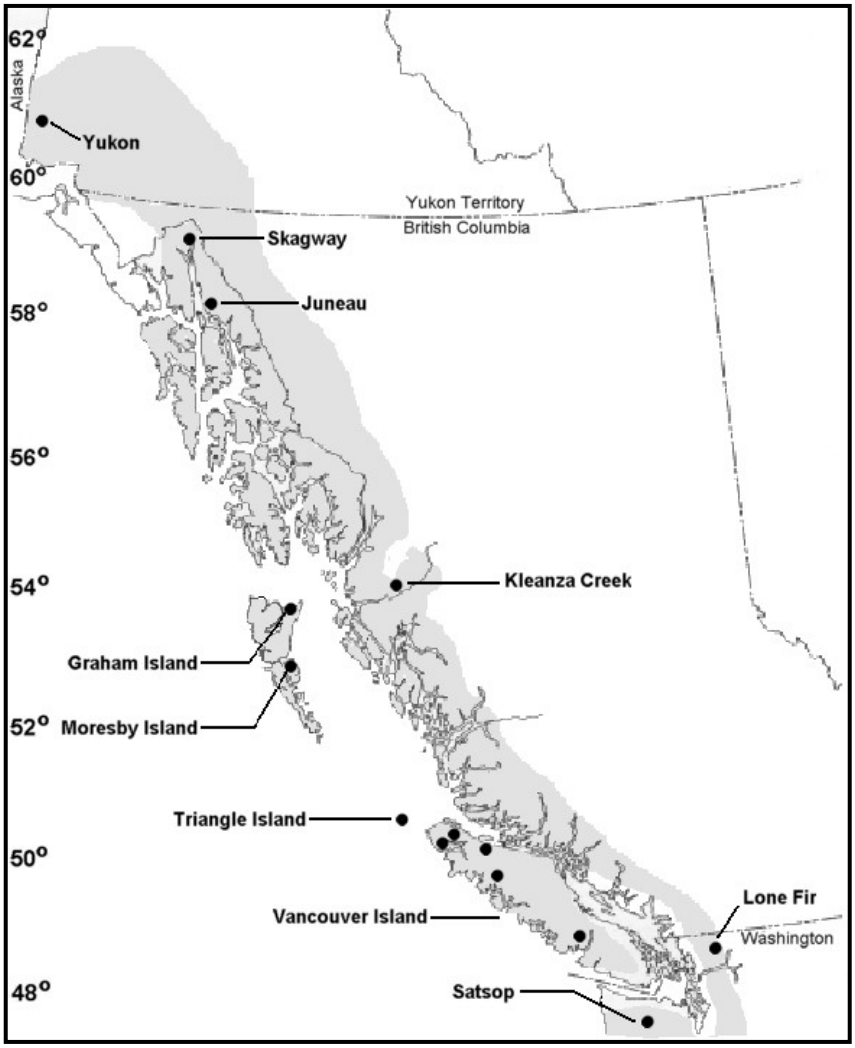


FIG. 9.–The distribution of *Peromyscus keeni* and the localities examined for this species.

maniculatus, in the mid to late Pleistocene (Hogan et al. 1993, 1997; Zheng et al. 2003). Preliminary analyses of the ND3/ND4L/ND4 region of the mitochondrial genome indicate 0.82% sequence variation within *P. keeni* and 4.4% sequence divergence from populations of *P. maniculatus* in the PNW (Hogan et al. 1997). Two studies of sequence variation at the control and/or cytochrome-b regions of mtDNA have addressed questions concerning the phylogeography of portions of the range of *P. keeni*. Concentrating on populations represented by small numbers of individuals ($n \leq 5$) from Washington, southern British Columbia and Vancouver Island, Zheng et al. (2003) suggested refugia for *P. keeni* on Vancouver Island and southern mainland British Columbia. From slightly larger samples ($n \leq 10$) from islands in the Alexander Archipelago and the adjacent mainland, Lucid and Cook (2004) concluded that *P. keeni* of these coastal islands have been influenced more by vicariance than by post-glacial dispersal and suggested that the relevant refugium was somewhere in this southeastern coastal region of southeastern Alaska. Neither of these studies, however, included populations of *P. keeni* from oceanic islands and/or a broad geographic sampling of coastal and mainland populations.

If the oceanic island populations of *P. keeni* were founded by dispersal from an interior mainland refugium, they should (due to founder effect and genetic drift) exhibit substantially less mtDNA sequence variation and haplotype diversity than their counterparts on the mainland. Additionally, the haplotypes present in oceanic island populations should be a subset of those present in the mainland populations. Further, if the oceanic insular populations of *P. keeni* were founded from mainland populations,

phylogenetic analyses of the mtDNA haplotypes should cluster these island populations within or among the mainland populations, and phylogeographic analysis would be expected to implicate contiguous range expansion from a mainland source. Alternatively, if the current distribution of *P. keeni* derived from a coastal (i.e. non-interior) refugium, the oceanic island populations are expected to exhibit suites of haplotypes distinctive from those on the mainland and, due to radiation from a centrally-located source, to demonstrate haplotypic diversity equal to or exceeding that seen in mainland populations.

Herein, I describe and compare the genetic variation in the mitochondrial ND3/ND4L/ND4 genes of mainland and coastal and oceanic insular populations of *P. keeni*, and assess the geographic partitioning of the resultant haplotypes. Given the expectations of the alternate phylogeographic hypotheses discussed above, analyses of haplotypic diversity and the extent of genetic and geographic partitioning were used to elucidate the biogeographic history of this species and provide evidence for the location of the refugium that gave rise to the current distribution of *P. keeni*.

Methods

Qiagen purification kits and protocols (Qiagen, Inc.) were used to isolate DNA from frozen (-80^{o}C) liver or spleen samples of 277 specimens of *P. keeni* from 24 (11 island and 13 mainland) localities (see Fig. 8 and Appendix A). Techniques for DNA

amplification and for obtaining sequences were as described in Chapter II. Each unique sequence was scored as an individual haplotype.

GenBank accession numbers for ND3/ND4L/ND4 sequences from populations of *P. keeni* were reported by Hogan et al. (1997). The animal use protocol employed in this study was conducted in accordance with the Guide for Care and Use of Laboratory Animals and was approved by the Texas A&M University Laboratory Animal Care and Use Committee.

Sequences of haplotypes were compared and their within- and among-locality variation was analyzed. Haplotype and nucleotide diversity as well as number of nucleotide substitutions per site were evaluated using MacClade Version 4.0 (Maddison and Maddison 2000). Neighbor-joining (NJ), maximum-parsimony (MP) and maximum likelihood (ML) analyses were conducted in PAUP*4.0b10 (Swofford 2002). Pairwise distances were computed using the uncorrected ("p") distance (Swofford 2002) and employed in NJ analyses. Maximum parsimony analyses of the sequence data consisted of heuristic searches using TBR branch swapping with equal weights and 10 random additions. Modeltest 3.06 (Posada and Crandall 1998) identified GTR+I+G (General Time Reversible + invariant sites + gamma distribution for variable sites, $\alpha = 0.8290$; $p_{inv} = 0.6855$) as the most appropriate model of nucleotide evolution for the data (applied in NJ (supplemental to "p" distance) and ML analyses). ML analysis was performed as in Chapter II. All analyses included reference sequences (Hogan et al. 1997, Chirhart et al. 2001) for single individuals of *P. maniculatus rufinus* (Colorado, GenBank Accession U40250) and *P. m. austerus* (Washingon, GenBank Accession U40249); sequence data

for *P. melanotis* (Hogan et al. 1997, Chirhart et al. 2001) from Hidalgo, Mexico (GenBank Accession U40247) were used as the outgroup for all phylogenetic analyses. Bootstrap estimates (Felsenstein 1985) based on 1000 replications were obtained for NJ, MP and ML analysis.

Results

Thirty-seven haplotypes were observed (Table 3) with only one individual from Vancouver Island sharing the most common haplotype from Triangle Island. All other haplotypes were unique to their respective populations. With the exception that there was only a single haplotype for the 20 mice from Moresby Island, neither the number of haplotypes nor haplotypic diversity was higher in the oceanic islands than in the populations from either Vancouver Island or the mainland. The unique suites of haplotypes characterizing each of the four insular populations (Graham Is., Moresby Is., Triangle Is., Vancouver Is.) differed by a mean of 0.9%. The five populations from the mainland exhibited a total of 22 different haplotypes with a mean divergence of 0.64%. The uncorrected ("p") distance between the sequences of insular and those of continental populations of *P. keeni* was 0.83%. Variation within the coding region of all sequences (excluding outgroup haplotypes) comprised a total of 142 transitions and 27 transversions, of which 107 (45.73%) occurred at third, 61 (26.07%) at first, and 66

Table 3

The number of haplotypes and percent sequence divergence within localities of *P. keeni*.

Locality	# Haplotypes	% Divergence
Graham Island	4	0.33
Moresby Island	1	0.00
Vancouver Island	6	0.16
Triangle Island	4	0.15
Kleanza Creek	5	0.15
Satsop, WA	3	0.14
Lone Fir, WA	5	0.31
SE Alaska	5	0.17
Yukon	4	0.12

(28.2%) at second codon positions. The nucleotide frequencies were 34.4%A, 23.8%C, 10.3%G, 31.4%T.

NJ distances based on both "p" and the GTR model and MP analyses identified the same clades (Figs. 10 & 11). The ML bootstrap consensus tree failed to resolve any major clades within *P. keeni*. Haplotypes for the mainland and Vancouver Island populations formed an internal cluster, whereas those for the oceanic islands (Graham Is., Moresby Is., Triangle Is.) grouped external and basal to the mainland/Vancouver Island associations. Haplotypes from the populations in Washington nested between these two clusters (Figs. 10 & 11).

Discussion

Geological (orogenic) and climatological (namely glacial) events during the late Tertiary and Quarternary Periods have caused complex distributional patterns within species, resulting in increased genetic diversity among populations of species in the PNW. Subsequently, at least three major distributional patterns characterize these taxa: 1) a disjunct distribution of species adapted to mesic forests of the Pacific coastal and Northern Rocky Mountain ranges, 2) a distributional pattern marked by a northern or southern range limit at the interface between the Cascade and Sierran Mountain ranges, and 3) a species distribution restricted to the mesic forests of the Northern Rocky Mountains (Brunsfeld et al. 2001). The geographic distribution of *P. keeni* is exemplary of the Cascade/Sierran pattern, and the current genetic diversity observed in this species

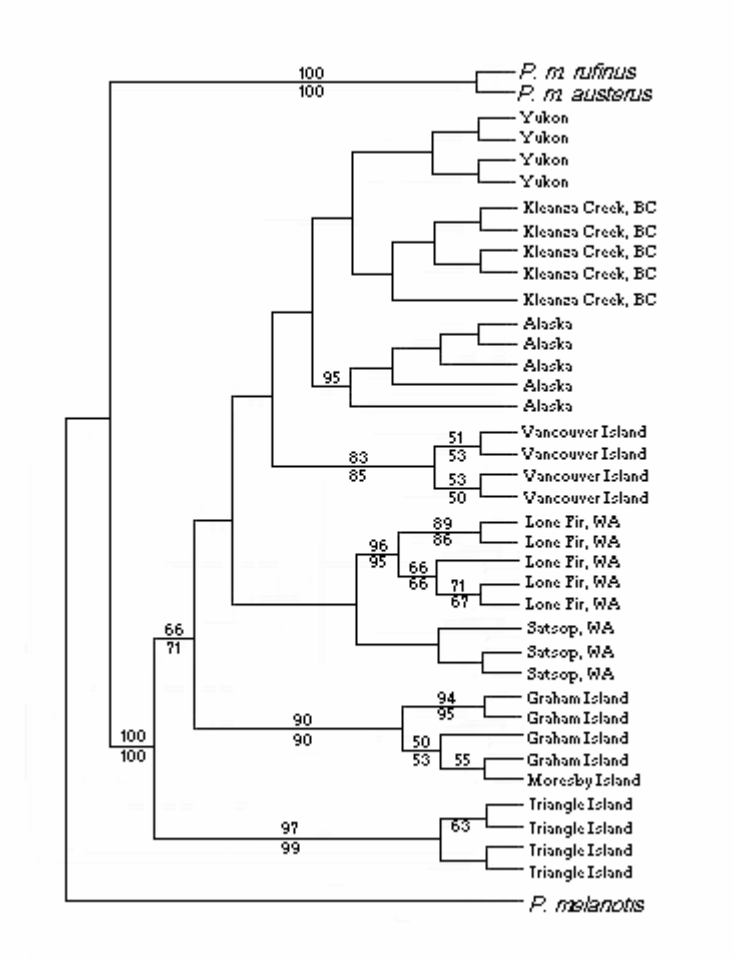


FIG. 10.–Neighbor-joining tree of uncorrected "p" distances. Numbers above lines = NJ bootstrap values. Numbers below lines = Strict consensus MP bootstrap values (both based on 1000 replications).

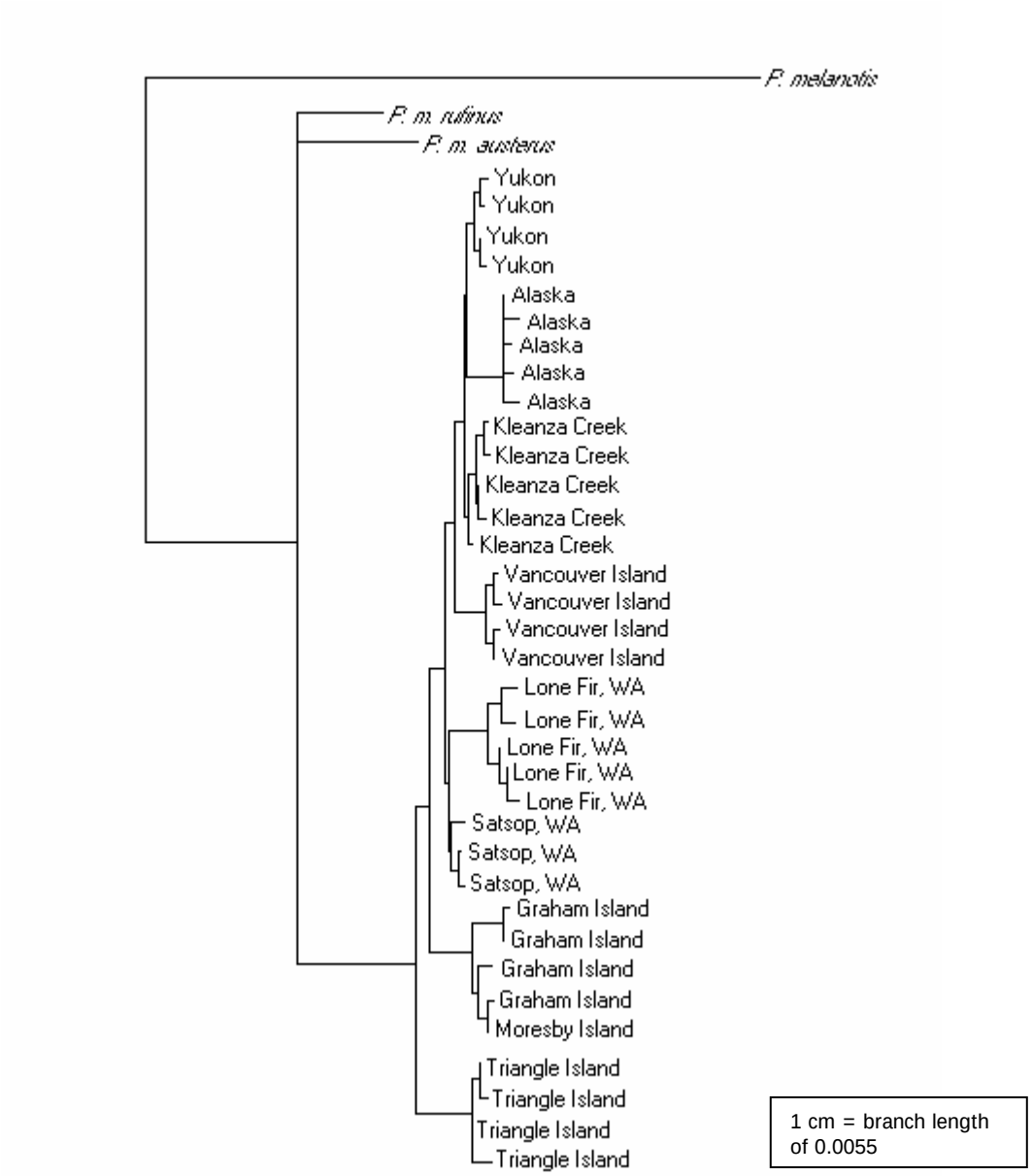
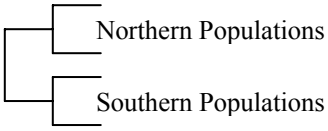


FIG. 11.–Neighbor-joining tree based on GTR model of nucleotide evolution, showing branch lengths among OTUs.

may in turn be explained by one of three associated phylogeographic hypotheses advanced by Brunsfeld et al. (2001). The Clinal Environment Hypothesis invokes isolation of geographically extreme populations by spatial and genetic distance due to a Cenozoic climatic cline. Under this hypothesis, widespread coastal species should exhibit gradual shifts in the frequencies of neutral nuclear markers, and variation in cytoplasmic genomes would not show concordance across multiple species. The Single Pacific Coast Refugium Hypothesis predicts a star phylogeny with very recent coalescence times (i.e. short internal branches) if the refugium was small. Species conforming to this hypothesis would display genetic uniformity as a result of common descent from a single refugial population, and neither north–south patterns nor concordance among species would be expected. Finally, the Multiple Refugia Hypotheses assumes one of two phylogenetic forms, the North-South Recolonization pattern or the Multiple Coastal Refugia pattern (Fig. 12).

The North-South Recolonization subhypothesis implies two refugia, one coastal, possibly in the vicinity of Vancouver Island or the Queen Charlotte Islands, and one (a nunatak) in the mountains south of glaciation (e.g. in the Siskiyous). Accordingly, patterns of genetic divergence should be partitioned into northern and southern clades. In addition, coalescence times would likely be short, and geographical concordance among codistributed taxa would be evident. The Multiple Coastal subhypothesis suggests that there are more than two refugia distributed along the coast and lowlands west of the Cascades. Multiple reciprocally monophyletic clades would exist, within

a. North-South Recolonization



b. Multiple Coastal Refugia

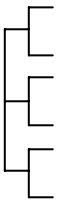


FIG. 12.–Subhypotheses of the Multiple Coastal Refugium Hypothesis advanced by Brunsfeld et al. (2001) to explain the Cascade/Sierran distributional pattern.

which coalescence times would be short. Relationships among these clades would perhaps be star-like and this pattern should be evident across other species.

Peromyscus keeni shares its Cascade/Sierran pattern of genetic differentiation with various gymnosperms, angiosperms, insects, teleost fishes, amphibians, and other mammals (Table 15.2 of Brunsfeld et al. 2001). However, contrary to the pattern observed in most of these codistributed plant and animal taxa (Soltis et al. 1997, Brown et al. 1997, Hatch 1990, Good 1989, Howard et al. 1993, Demboski and Cook 2001), mtDNA sequences of populations from *P. keeni* indicate a lack of north-south geographic structure. These data for *P. keeni* conform best to Brunsfeld's (2001) Single Pacific Coast Refugium Hypothesis, as the populations are relatively genetically uniform (overall sequence divergence of 0.82%) and are characterized by short branch lengths (Fig. 11, average # of steps per character in the neighbor-joining tree = 0.163). Surveys of genetic variation in sticklebacks (*Gasterosteus aculeatus,* O'Reilly et al. 1993), song sparrows (*Melospiza Melodia*, Zink and Ditmann 1993) and black bears (*Ursus americanus*, Byun et al. 1997) suggest that the Queen Charlotte Islands represent a vital Pleistocene glacial refugium (the Haida Gwaii refugium) for animal taxa, though the existence of other refugia for these species has been suggested as equally likely (Orti et al. 1994, Demboski et al.1998).

Despite their conspicuous lack of insular samples of *P. keeni* (other than Vancouver Island, which is effectively a mainland zone) and samples north of Vancouver Island, Zheng et al. (2003) suggested that a refugium for *P. keeni* existed on Vancouver Island and southern continental British Columbia. Due to sample limitations,

however, the authors could not preclude the option of a more northern or non-mainland refugium. Lucid and Cook (2004), as a result of their sampling of coastal insular and mainland populations of *P. keeni* in southeast Alaska, contended that the refugium was present in that region. As the individuals analyzed herein represent mainland and oceanic island populations of *P. keeni* from Washington northward to the Yukon Territory, geographic sampling bias has less influence on our phylogeographic hypothesis, specifically as it pertains to an initial refugium pertinent to the speciation of *P. keeni*. Contrary to the population genetic predictions for island populations derived from mainland sources, haplotype variation (numbers of haplotypes) and diversity (amount of sequence difference between haplotypes) observed in *P. keeni* were equally high in the oceanic island and mainland populations (Table 3). Furthermore, haplotypes in populations from the oceanic Triangle and Queen Charlotte Islands grouped outside of the mainland and Vancouver Island cluster (Figs. 10 & 11). Thus, the mtDNA-based phylogeography is clearly inconsistent with the genetic expectations of the mainland refugium/standard island biogeography hypothesis for the overall distribution of *P. keeni*. The data presented herein are, however, consistent with the Hecate refugium hypothesis (Byun et al. 1997, 1999), which states that the relevant ice-free area persisted on the now submerged continental shelf separating the Queen Charlotte Islands from the mainland. Byun et al. (1999) suggested that recent sonar data and sedimentary cores (Barrie et al. 1993; Josenhans et al. 1993, 1995) provide "physical proof" that the continental shelf beneath the Hecate Strait was terrestrial, ice-free and dotted with freshwater lakes during the Wisconsinan glacial maximum (~15,000 ya). Post-

Pleistocene terrestrial dispersal from a geographically intermediate zone such as a Hecate refugium would be expected to result in genetic uniformity among mainland, coastal and oceanic insular populations. If *P. keeni* was initially isolated in a Hecate refugium, the island populations would represent relicts of the ancestral population forced onto unglaciated higher ground as the melting ice resulted in sea level transgression. In this case, island populations would not be expected to exhibit low levels of genetic variation, and would be phylogenetically basal to continental populations, displaying a phylogeographic pattern that lacks concordance over large geographic distances. The mtDNA variation observed in this study conforms to this pattern, and *P. keeni* therefore provides a model taxon against which to test phylogeographic hypotheses concerning geographical concordance as a result of Pleistocene isolation in a Hecate refugium.

CHAPTER V

SUMMARY AND CONCLUSIONS

Two species in the *Peromyscus maniculatus* species group occur along the west coast of North America: 1) *P. keeni*, a terrestrial species occurring in the coastal deciduous forests and on numerous coastal and isolated oceanic islands in the Pacific Northwest, and 2) *P. maniculatus*, which is distributed from the Atlantic to Pacific seaboards and from the Northwest Territory to Panama. A third species in this group, *P. sejugis* (restricted to two arid islands in the Sea of Cortés, Isla San Diego and Isla Santa Cruz), has also been inexorably affected by west coast vicariant events. Herein, I summarize the geologic history of this region, as well as its effects on the species in this complex, from south to north, beginning with Baja California and its associated islands.

The present-day biota of mainland Baja California is thought to have arisen via a series of vicariant events during the late Neogene (5.5-1 mya, Murphy 1983; Grismer 1994; Riddle 1995; Riddle et al. 2000a,b,c; Carreño and Helenes 2002). The Sea of Cortés is purported to have formed in the late Neogene (more specifically during the Pliocene, 3 to 4mybp), as Baja California began to rift apart from the Mexican mainland (Harden 1998). The successive riftings that generated the arid islands in this sea likely led to fragmentation of the mainland arid-adapted Pliocene and Pleistocene faunas of Baja California. Correspondingly, these islands are inhabited primarily by desert-adapted mammals with sister taxa on the proximal mainland (Lawlor et al. 2002), as

seen in the distributions of *P. sejugis* and *P. maniculatus*, respectively. Specifically, islands in the archipelago including Isla Espiritu Santo and Isla San Jose, and thus presumably Isla San Diego and Isla Santa Cruz, were severed from the present-day La Paz Peninsula during a Pleistocene glacial advance 25,000-17,000 years ago (Beal 1948). This potentially fragmented a formerly contiguous *Peromyscus maniculatus* ancestral stock. Shortly (in a geologic sense) following fragmentation, gene flow and waif dispersal between Baja California and the islands would have been precluded by the geologic transgressions that ultimately resulted in the present sea level approximately 6,000 years ago (Avise et al. 1979). Over the course of at least the last 6,000 years, then, the insular endemic populations of *P. sejugis* apparently experienced independent evolutionary trajectories as a result of founder effect, genetic drift and inbreeding. This is manifest in differential mtDNA haplotypes as well as fixed allozymic, chromosomal, microsatellite, and morphological characters diagnostic of each insular population of *P. sejugis* relative to the *P. maniculatus* from peninsular Baja California. Such genealogical concordance potentially supports the validity of each population of *P. sejugis* as a phylogenetic species, and at least documents the current disposition of each of these populations as a distinct evolutionarily significant unit.

The majority of major orogenies effecting modern day California occurred 3 to 5 mybp (early to mid Pliocene) in response to increased compression along the boundary of the Pacific and North American tectonic plates (Harden 1998). Included was the formation of the Sierra Nevada mountain range, which acts as a barrier to gene flow between terrestrial animals including coastal California populations of *P. maniculatus*

and those from central North America, between ~35 and 39^{o} North latitude. This lack of gene flow has contributed to the ostensible establishment of a cryptic species of *Peromyscus* extending from the southern tip of Baja California to a region between Fresno and Arcata, CA, presumably at the San Francisco Bay/Sacramento-San Joaquin Delta area. This currently unrecognized Baja/S California species is phylogenetically closer to *P. sejugis* than it is to continental *P. maniculatus*. The geographic separation of clades of *Neotoma fuscipes* (the dusky-footed woodrat; Hooper 1944, Matocq 2002) and a number of other rodents (Hooper 1944) and other terrestrial species at the San Francisco Bay area is due to climatic and habitat differences as well as glaciation of river drainages in northwestern California during the Pleistocene (~12,000ybp). Such geographical concordance among co-distributed species corroborates the division of a central and northern U.S./northern California clade of *P. maniculatus* from the Baja/southwestern and central California *P. maniculatus* clade. This shared pattern lends geographic validity to the genealogical concordance of mtDNA (both RFLP and sequence data) and morphology to the hypothesis of the existence of a cryptic Baja/S California species within what is currently recognized as *P. maniculatus*. The existence of a peripheral isolate species in the Baja/Southern California region is also consistent with the overall geographic distribution of the *P. maniculatus* group species including well-recognized peripheral isolate species in the northwest (*P. keeni*), southeast (*P. polionotus*) and south-central (*P. melanotis*) regions of North America. It is likely that the events that led to each of these peripheral isolate species is related to the geologic events associated with the glaciations of the Pleistocene.

The Pacific Northwest of North America, like the west coast of the United States, is generally characterized by the presence of "coastal" species whose sister taxon is a more widely distributed "mainland" species (e.g. *P. keeni* and *P. maniculatus*, respectively). In fact, *P. keeni* is thought to have diverged from *P. maniculatus*, in the mid to late Pleistocene (Hibbard 1968). This distributional relationship has been hypothesized to be the result of the colonization of Holocene insular zones from an ice-free coastal refugium in mainland British Columbia or Washington following the Wisconsinan (late Pleistocene) glacial retreat (Demboski et al. 1999). However, 2 coastal refugia and 13 nunataks (mountain refugia) flanked the Cordilleran ice sheet ~13,000 ago, including one on Vancouver Island and one on the Queen Charlotte Islands (Graham and Moresby Islands; Pielou 1991). It is thought that one or both of these refugia, and/or a reputed refugium in the present-day Hecate Strait (between the Queen Charlottes and mainland British Columbia) served as a sanctuary for many Pleistocene organisms. The latter refugium ostensibly (based on the pattern of mtDNA sequence variation described in this study) accommodated the ancestor to *P. keeni* throughout the height of the Pleistocene glacial advance in North America. At approximately 10,000 ybp, much of the PNW coast was still isolated by ice from the remainder of Canada, allowing for the establishment of disparate habitat to which coastal species evolved. These species, including *P. keeni*, were then inextricably tied to these habitats and remain as the present-day "coastal" species of the Pacific Northwest of North America.

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APPENDIX A

SPECIMENS EXAMINED

BS = Baja California del Sur, BN= Baja Calif. del Norte, BC = British Columbia, YK = Yukon, VC = Vancouver Isl., GI = Graham Isl., MI = Moresby Island, TI = Triangle Island, GK = Greenbaum Karyotype number, NK = New Mexico Karyotype number, LVT = Tissue obtained from Brett Riddle, University of Nevada, Las Vegas

ID number	*Genus species*	County or Region	State
GK 5462	*P. sejugis*	Isla San Diego	BS
5463	*P. sejugis*	Isla San Diego	BS
5464	*P. sejugis*	Isla San Diego	BS
5465	*P. sejugis*	Isla San Diego	BS
5466	*P. sejugis*	Isla San Diego	BS
5467	*P. sejugis*	Isla San Diego	BS
5468	*P. sejugis*	Isla San Diego	BS
5469	*P. sejugis*	Isla San Diego	BS
5470	*P. sejugis*	Isla San Diego	BS
5471	*P. sejugis*	Isla San Diego	BS
5472	*P. sejugis*	Isla San Diego	BS
5473	*P. sejugis*	Isla San Diego	BS
5474	*P. sejugis*	Isla San Diego	BS
5477	*P. sejugis*	Isla Santa Cruz	BS
5478	*P. sejugis*	Isla Santa Cruz	BS
5479	*P. sejugis*	Isla Santa Cruz	BS
5480	*P. sejugis*	Isla Santa Cruz	BS
5481	*P. sejugis*	Isla Santa Cruz	BS
5482	*P. sejugis*	Isla Santa Cruz	BS
5483	*P. sejugis*	Isla Santa Cruz	BS
NK 288	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
290	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
291	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
292	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
293	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
294	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
296	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
298	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
299	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
300	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
301	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
302	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
303	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
305	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
306	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
307	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
308	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
327	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
4607	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
4613	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
5479	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN

5480	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
5481	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
5482	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
5483	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
5484	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
5486	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
5494	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
LVT 03693	*P. maniculatus*	Laguna Hanson, Sierra Juarez	BN
NK 5398	*P. maniculatus*	Vallecitos	BN
5399	*P. maniculatus*	Vallecitos	BN
5413	*P. maniculatus*	Vallecitos	BN
5414	*P. maniculatus*	Vallecitos	BN
5420	*P. maniculatus*	Vallecitos	BN
5435	*P. maniculatus*	Vallecitos	BN
5436	*P. maniculatus*	Vallecitos	BN
5439	*P. maniculatus*	Vallecitos	BN
5440	*P. maniculatus*	Vallecitos	BN
5441	*P. maniculatus*	Vallecitos	BN
5444	*P. maniculatus*	Vallecitos	BN
5446	*P. maniculatus*	Vallecitos	BN
5447	*P. maniculatus*	Vallecitos	BN
5448	*P. maniculatus*	Vallecitos	BN
5449	*P. maniculatus*	Vallecitos	BN
5450	*P. maniculatus*	Vallecitos	BN
5452	*P. maniculatus*	Vallecitos	BN
5457	*P. maniculatus*	Vallecitos	BN
5458	*P. maniculatus*	Vallecitos	BN
5459	*P. maniculatus*	Vallecitos	BN
5464	*P. maniculatus*	Vallecitos	BN
5465	*P. maniculatus*	Vallecitos	BN
5466	*P. maniculatus*	Vallecitos	BN
5467	*P. maniculatus*	Vallecitos	BN
5468	*P. maniculatus*	Vallecitos	BN
5474	*P. maniculatus*	Vallecitos	BN
NK 122	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
124	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
126	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
130	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
133	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
135	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
139	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
141	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
142	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
143	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
148	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
194	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
195	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
197	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
214	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
221	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
222	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
223	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
224	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN

225	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
226	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
229	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
230	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
231	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
232	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
248	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
249	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
253	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
LVT 03793	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
LVT 03800	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
LVT 03801	*P. maniculatus*	Valle de Trinidad, Sierra de San Pedro Martir	BN
NK 5387	*P. maniculatus*	Colonio Vicente Guerrero	BN
5388	*P. maniculatus*	Colonio Vicente Guerrero	BN
5389	*P. maniculatus*	Colonio Vicente Guerrero	BN
LVT 03752	*P. maniculatus*	Mision San Fernando	BN
LVT 02192	*P. maniculatus*	27 km S Punta Prieta	BN
02193	*P. maniculatus*	28 km S Punta Prieta	BN
02194	*P. maniculatus*	29 km S Punta Prieta	BN
02195	*P. maniculatus*	30 km S Punta Prieta	BN
NK 5147	*P. maniculatus*	Guerrero Negro	BS
LVT 03634	*P. maniculatus*	Todos Santos	BS
CP 1	*P. maniculatus*	Oceanside, San Diego Co.	CA
2	*P. maniculatus*	Oceanside, San Diego Co.	CA
3	*P. maniculatus*	Oceanside, San Diego Co.	CA
4	*P. maniculatus*	Oceanside, San Diego Co.	CA
5	*P. maniculatus*	Oceanside, San Diego Co.	CA
6	*P. maniculatus*	Oceanside, San Diego Co.	CA
7	*P. maniculatus*	Oceanside, San Diego Co.	CA
8	*P. maniculatus*	Oceanside, San Diego Co.	CA
9	*P. maniculatus*	Oceanside, San Diego Co.	CA
10	*P. maniculatus*	Oceanside, San Diego Co.	CA
11	*P. maniculatus*	Oceanside, San Diego Co.	CA
12	*P. maniculatus*	Oceanside, San Diego Co.	CA
13	*P. maniculatus*	Oceanside, San Diego Co.	CA
14	*P. maniculatus*	Oceanside, San Diego Co.	CA
15	*P. maniculatus*	Oceanside, San Diego Co.	CA
16	*P. maniculatus*	Oceanside, San Diego Co.	CA
17	*P. maniculatus*	Oceanside, San Diego Co.	CA
18	*P. maniculatus*	Oceanside, San Diego Co.	CA
19	*P. maniculatus*	Oceanside, San Diego Co.	CA
20	*P. maniculatus*	Oceanside, San Diego Co.	CA
21	*P. maniculatus*	Oceanside, San Diego Co.	CA
22	*P. maniculatus*	Oceanside, San Diego Co.	CA
NK 357	*P. maniculatus*	Boulder Bay, San Bernadino Co.	CA
358	*P. maniculatus*	Boulder Bay, San Bernadino Co.	CA
359	*P. maniculatus*	Boulder Bay, San Bernadino Co.	CA
NK 4726	*P. maniculatus*	Big Bear City, San Bernadino Co.	CA
4727	*P. maniculatus*	Big Bear City, San Bernadino Co.	CA
4728	*P. maniculatus*	Big Bear City, San Bernadino Co.	CA
4732	*P. maniculatus*	Big Bear City, San Bernadino Co.	CA
4733	*P. maniculatus*	Big Bear City, San Bernadino Co.	CA
4734	*P. maniculatus*	Big Bear City, San Bernadino Co.	CA

4735	*P. maniculatus*	Big Bear City, San Bernadino Co.	CA
4736	*P. maniculatus*	Big Bear City, San Bernadino Co.	CA
4737	*P. maniculatus*	Big Bear City, San Bernadino Co.	CA
4738	*P. maniculatus*	Big Bear City, San Bernadino Co.	CA
NK 349	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
352	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
353	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
354	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
355	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
356	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
363	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
364	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
365	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
366	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
368	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
369	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
370	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
371	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
373	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
374	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
376	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
377	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
378	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
379	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
380	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
391	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
392	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
393	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
400	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
401	*P. maniculatus*	Heart Bar, San Bernadino Co.	CA
NK 421	*P. maniculatus*	Johannesburg, San Bernadino Co.	CA
422	*P. maniculatus*	Johannesburg, San Bernadino Co.	CA
423	*P. maniculatus*	Johannesburg, San Bernadino Co.	CA
483	*P. maniculatus*	Johannesburg, San Bernadino Co.	CA
484	*P. maniculatus*	Johannesburg, San Bernadino Co.	CA
NK 514	*P. maniculatus*	Fresno, Madera Co.	CA
516	*P. maniculatus*	Fresno, Madera Co.	CA
517	*P. maniculatus*	Fresno, Madera Co.	CA
518	*P. maniculatus*	Fresno, Madera Co.	CA
519	*P. maniculatus*	Fresno, Madera Co.	CA
522	*P. maniculatus*	Fresno, Madera Co.	CA
542	*P. maniculatus*	Fresno, Madera Co.	CA
543	*P. maniculatus*	Fresno, Madera Co.	CA
544	*P. maniculatus*	Fresno, Madera Co.	CA
545	*P. maniculatus*	Fresno, Madera Co.	CA
546	*P. maniculatus*	Fresno, Madera Co.	CA
547	*P. maniculatus*	Fresno, Madera Co.	CA
548	*P. maniculatus*	Fresno, Madera Co.	CA
552	*P. maniculatus*	Fresno, Madera Co.	CA
554	*P. maniculatus*	Fresno, Madera Co.	CA
555	*P. maniculatus*	Fresno, Madera Co.	CA
562	*P. maniculatus*	Fresno, Madera Co.	CA
563	*P. maniculatus*	Fresno, Madera Co.	CA

564	*P. maniculatus*	Fresno, Madera Co.	CA
565	*P. maniculatus*	Fresno, Madera Co.	CA
568	*P. maniculatus*	Fresno, Madera Co.	CA
570	*P. maniculatus*	Fresno, Madera Co.	CA
NK 596	*P. maniculatus*	Arcata, Humboldt Co.	CA
608	*P. maniculatus*	Arcata, Humboldt Co.	CA
609	*P. maniculatus*	Arcata, Humboldt Co.	CA
610	*P. maniculatus*	Arcata, Humboldt Co.	CA
612	*P. maniculatus*	Arcata, Humboldt Co.	CA
622	*P. maniculatus*	Arcata, Humboldt Co.	CA
626	*P. maniculatus*	Arcata, Humboldt Co.	CA
627	*P. maniculatus*	Arcata, Humboldt Co.	CA
628	*P. maniculatus*	Arcata, Humboldt Co.	CA
629	*P. maniculatus*	Arcata, Humboldt Co.	CA
633	*P. maniculatus*	Arcata, Humboldt Co.	CA
NK 660	*P. maniculatus*	Burns, Harney Co.	OR
661	*P. maniculatus*	Burns, Harney Co.	OR
662	*P. maniculatus*	Burns, Harney Co.	OR
663	*P. maniculatus*	Burns, Harney Co.	OR
664	*P. maniculatus*	Burns, Harney Co.	OR
665	*P. maniculatus*	Burns, Harney Co.	OR
666	*P. maniculatus*	Burns, Harney Co.	OR
667	*P. maniculatus*	Burns, Harney Co.	OR
668	*P. maniculatus*	Burns, Harney Co.	OR
669	*P. maniculatus*	Burns, Harney Co.	OR
670	*P. maniculatus*	Burns, Harney Co.	OR
671	*P. maniculatus*	Burns, Harney Co.	OR
672	*P. maniculatus*	Burns, Harney Co.	OR
673	*P. maniculatus*	Burns, Harney Co.	OR
674	*P. maniculatus*	Burns, Harney Co.	OR
675	*P. maniculatus*	Burns, Harney Co.	OR
676	*P. maniculatus*	Burns, Harney Co.	OR
677	*P. maniculatus*	Burns, Harney Co.	OR
678	*P. maniculatus*	Burns, Harney Co.	OR
679	*P. maniculatus*	Burns, Harney Co.	OR
NK 707	*P. maniculatus*	Alsea, Benton Co.	OR
709	*P. maniculatus*	Alsea, Benton Co.	OR
717	*P. maniculatus*	Alsea, Benton Co.	OR
718	*P. maniculatus*	Alsea, Benton Co.	OR
719	*P. maniculatus*	Alsea, Benton Co.	OR
720	*P. maniculatus*	Alsea, Benton Co.	OR
721	*P. maniculatus*	Alsea, Benton Co.	OR
722	*P. maniculatus*	Alsea, Benton Co.	OR
723	*P. maniculatus*	Alsea, Benton Co.	OR
724	*P. maniculatus*	Alsea, Benton Co.	OR
725	*P. maniculatus*	Alsea, Benton Co.	OR
726	*P. maniculatus*	Alsea, Benton Co.	OR
727	*P. maniculatus*	Alsea, Benton Co.	OR
728	*P. maniculatus*	Alsea, Benton Co.	OR
729	*P. maniculatus*	Alsea, Benton Co.	OR
730	*P. maniculatus*	Alsea, Benton Co.	OR
731	*P. maniculatus*	Alsea, Benton Co.	OR
732	*P. maniculatus*	Alsea, Benton Co.	OR

733	*P. maniculatus*	Alsea, Benton Co.	OR
GK 5711	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5712	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5765	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5766	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5767	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5769	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5770	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5771	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5772	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5773	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5774	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5775	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5779	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5780	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5781	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5782	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5783	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
5784	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
6033	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
6034	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
6039	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
6040	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
6041	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
6048	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
6049	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
6050	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
6051	*P. maniculatus*	Varden Creek, Okanogan Co.	WA
GK 1951	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
5395	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
5402	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
5409	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
5414	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
5415	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
5909	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6110	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6111	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6132	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6143	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6145	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6159	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6197	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6210	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6225	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6228	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6247	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6253	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6262	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6263	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6264	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6274	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6275	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6318	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA

6328	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6329	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6330	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6445	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6446	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6447	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6455	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6458	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6459	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6473	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6519	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6522	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6523	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6582	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6583	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6584	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6585	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6597	*P. maniculatus*	Satsop, Gray's Harbor Co.	WA
6087	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6088	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6094	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6124	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6125	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6196	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6215	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6216	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6217	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6218	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6240	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6241	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6244	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6245	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6246	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6271	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6272	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6281	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6284	*P. keeni*	Satsop, Gray's Harbor Co.	WA
6312	*P. keeni*	Satsop, Gray's Harbor Co.	WA
5689	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5690	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5691	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5692	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5693	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5694	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5695	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5696	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5697	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5698	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5699	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5700	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5701	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5702	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5703	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA

5704	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5705	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5706	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5707	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6114	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6058	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6059	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6060	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6061	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6085	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6086	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6204	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6205	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6206	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6207	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6211	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6212	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6213	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
6214	*P. keeni*	Lone Fir Cmpgrd, Okanogan Co.	WA
5721	*P. keeni*	Silver Star Creek, Okanogan Co.	WA
5722	*P. keeni*	Silver Star Creek, Okanogan Co.	WA
5723	*P. keeni*	Silver Star Creek, Okanogan Co.	WA
5724	*P. keeni*	Silver Star Creek, Okanogan Co.	WA
5725	*P. keeni*	Silver Star Creek, Okanogan Co.	WA
5726	*P. keeni*	Silver Star Creek, Okanogan Co.	WA
1974	*P. keeni*	Kleanza Creek, Hwy 16	BC
1980	*P. keeni*	Kleanza Creek, Hwy 17	BC
1981	*P. keeni*	Kleanza Creek, Hwy 18	BC
1982	*P. keeni*	Kleanza Creek, Hwy 19	BC
1983	*P. keeni*	Kleanza Creek, Hwy 20	BC
1984	*P. keeni*	Kleanza Creek, Hwy 21	BC
1985	*P. keeni*	Kleanza Creek, Hwy 22	BC
1994	*P. keeni*	Kleanza Creek, Hwy 23	BC
2013	*P. keeni*	Kleanza Creek, Hwy 24	BC
2017	*P. keeni*	Kleanza Creek, Hwy 25	BC
2021	*P. keeni*	Kleanza Creek, Hwy 26	BC
2022	*P. keeni*	Kleanza Creek, Hwy 27	BC
2154	*P. keeni*	Kleanza Creek, Hwy 28	BC
2155	*P. keeni*	Kleanza Creek, Hwy 29	BC
2156	*P. keeni*	Kleanza Creek, Hwy 30	BC
2157	*P. keeni*	Kleanza Creek, Hwy 31	BC
2158	*P. keeni*	Kleanza Creek, Hwy 32	BC
2159	*P. keeni*	Kleanza Creek, Hwy 33	BC
2160	*P. keeni*	Kleanza Creek, Hwy 34	BC
2161	*P. keeni*	Kleanza Creek, Hwy 35	BC
2162	*P. keeni*	Kleanza Creek, Hwy 36	BC
2163	*P. keeni*	Kleanza Creek, Hwy 37	BC
2164	*P. keeni*	Kleanza Creek, Hwy 38	BC
2165	*P. keeni*	Kleanza Creek, Hwy 39	BC
2166	*P. keeni*	Kleanza Creek, Hwy 40	BC
2206	*P. keeni*	Kleanza Creek, Hwy 41	BC
2216	*P. keeni*	Kleanza Creek, Hwy 42	BC
2245	*P. keeni*	Kleanza Creek, Hwy 43	BC

1986	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
1987	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
1988	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
1989	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
1990	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
1991	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
2012	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
2015	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
2018	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
2019	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
2020	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
2023	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
2169	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
2243	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
2247	*P. keeni*	4.6km W Exchamsiks R. Prov. Park	BC
1992	*P. keeni*	32.4km E Prince Rupert	BC
1993	*P. keeni*	32.4km E Prince Rupert	BC
2009	*P. keeni*	32.4km E Prince Rupert	BC
2010	*P. keeni*	32.4km E Prince Rupert	BC
2007	*P. keeni*	32.4km E Prince Rupert	BC
2008	*P. keeni*	32.4km E Prince Rupert	BC
2011	*P. keeni*	32.4km E Prince Rupert	BC
2230	*P. keeni*	16km S, 12 km E Hagensborg	BC
2304	*P. keeni*	vic Skagway, Liarsville Cmpgrd.	AK
2305	*P. keeni*	vic Skagway, Liarsville Cmpgrd.	AK
2306	*P. keeni*	vic Skagway, Liarsville Cmpgrd.	AK
2307	*P. keeni*	vic Skagway, Liarsville Cmpgrd.	AK
2308	*P. keeni*	vic Skagway, Liarsville Cmpgrd.	AK
2372	*P. keeni*	vic Skagway, Liarsville Cmpgrd.	AK
2373	*P. keeni*	vic Skagway, Liarsville Cmpgrd.	AK
2374	*P. keeni*	vic Skagway, Liarsville Cmpgrd.	AK
2375	*P. keeni*	vic Skagway, Liarsville Cmpgrd.	AK
2309	*P. keeni*	Auke Bay Cmpgrd, 1mi NW Ferry Terminal	AK
2310	*P. keeni*	Hebert R., 12.7mi NW Ferry Terminal	AK
2311	*P. keeni*	Hebert R., 12.7mi NW Ferry Terminal	AK
2312	*P. keeni*	Hebert R., 12.7mi NW Ferry Terminal	AK
2313	*P. keeni*	Hebert R., 12.7mi NW Ferry Terminal	AK
2314	*P. keeni*	Hebert R., 12.7mi NW Ferry Terminal	AK
2315	*P. keeni*	Hebert R., 12.7mi NW Ferry Terminal	AK
2316	*P. keeni*	Hebert R., 12.7mi NW Ferry Terminal	AK
2354	*P. keeni*	Hebert R., 12.7mi NW Ferry Terminal	AK
2355	*P. keeni*	Hebert R., 12.7mi NW Ferry Terminal	AK
2356	*P. keeni*	Hebert R., 12.7mi NW Ferry Terminal	AK
2357	*P. keeni*	Hebert R., 12.7mi NW Ferry Terminal	AK
2358	*P. keeni*	Hebert R., 12.7mi NW Ferry Terminal	AK
2359	*P. keeni*	Hebert R., 12.7mi NW Ferry Terminal	AK
2303	*P. keeni*	Kusawa Lake Cmpgrd	YK
2360	*P. keeni*	Kusawa Lake Cmpgrd	YK
2361	*P. keeni*	Kusawa Lake Cmpgrd	YK
6335	*P. keeni*	Kusawa Lake Cmpgrd	YK
6336	*P. keeni*	Kusawa Lake Cmpgrd	YK
6425	*P. keeni*	Kusawa Lake Cmpgrd	YK
6426	*P. keeni*	Kusawa Lake Cmpgrd	YK

6341	*P. keeni*	Kluane Park Reserve Farm	YK
6342	*P. keeni*	Kluane Park Reserve Farm	YK
6343	*P. keeni*	Kluane Park Reserve Farm	YK
6344	*P. keeni*	Kluane Park Reserve Farm	YK
6415	*P. keeni*	Kluane Park Reserve Farm	YK
6416	*P. keeni*	Kluane Park Reserve Farm	YK
6417	*P. keeni*	Kluane Park Reserve Farm	YK
6418	*P. keeni*	Kluane Park Reserve Farm	YK
6422	*P. keeni*	Kluane Park Reserve Farm	YK
6423	*P. keeni*	Kluane Park Reserve Farm	YK
6424	*P. keeni*	Kluane Park Reserve Farm	YK
6345	*P. keeni*	Kluane Park Maintenance Shed	YK
6405	*P. keeni*	Kluane Park Maintenance Shed	YK
6406	*P. keeni*	Kluane Park Maintenance Shed	YK
6407	*P. keeni*	Kluane Park Maintenance Shed	YK
6408	*P. keeni*	Kluane Park Maintenance Shed	YK
6409	*P. keeni*	Kluane Park Maintenance Shed	YK
6410	*P. keeni*	Kluane Park Maintenance Shed	YK
751	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
752	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
753	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
755	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
756	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
757	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
758	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
759	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
760	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
761	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
762	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
763	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
764	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
765	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
766	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
767	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
768	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
769	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
770	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
771	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
772	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
773	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
785	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
786	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
787	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
788	*P. keeni*	Pacific Rim Cmpgrd, 4.8mi S Tofino	VC
848	*P. keeni*	Rupert Inlet, 13.5mi W Port McNeill	VC
849	*P. keeni*	Rupert Inlet, 13.5mi W Port McNeill	VC
863	*P. keeni*	Rupert Inlet, 13.5mi W Port McNeill	VC
2447	*P. keeni*	Mt. Washington Ski Area	VC
2448	*P. keeni*	Mt. Washington Ski Area	VC
2449	*P. keeni*	Mt. Washington Ski Area	VC
6135	*P. keeni*	Mt. Washington Ski Area	VC
6136	*P. keeni*	Mt. Washington Ski Area	VC
6137	*P. keeni*	Mt. Washington Ski Area	VC

1716	*P. keeni*	Marble River Recreation Area	VC
1718	*P. keeni*	Marble River Recreation Area	VC
1719	*P. keeni*	Marble River Recreation Area	VC
1720	*P. keeni*	Marble River Recreation Area	VC
1721	*P. keeni*	Marble River Recreation Area	VC
1725	*P. keeni*	Marble River Recreation Area	VC
6000	*P. keeni*	Marble River Recreation Area	VC
6074	*P. keeni*	Marble River Recreation Area	VC
6078	*P. keeni*	Marble River Recreation Area	VC
6092	*P. keeni*	Marble River Recreation Area	VC
6194	*P. keeni*	Marble River Recreation Area	VC
1699	*P. keeni*	4.4 mi N Zeballos	VC
1700	*P. keeni*	4.4 mi N Zeballos	VC
1701	*P. keeni*	4.4 mi N Zeballos	VC
1709	*P. keeni*	4.4 mi N Zeballos	VC
1711	*P. keeni*	4.4 mi N Zeballos	VC
1998	*P. keeni*	Phantom Creek, 10km marker	GI
1999	*P. keeni*	Phantom Creek, 10km marker	GI
2000	*P. keeni*	Phantom Creek, 10km marker	GI
2001	*P. keeni*	Phantom Creek, 10km marker	GI
2002	*P. keeni*	Phantom Creek, 10km marker	GI
2004	*P. keeni*	Phantom Creek, 10km marker	GI
2005	*P. keeni*	Phantom Creek, 10km marker	GI
2006	*P. keeni*	Phantom Creek, 10km marker	GI
2067	*P. keeni*	Phantom Creek, 10km marker	GI
2068	*P. keeni*	Phantom Creek, 10km marker	GI
2069	*P. keeni*	Phantom Creek, 10km marker	GI
2070	*P. keeni*	Phantom Creek, 10km marker	GI
2071	*P. keeni*	Phantom Creek, 10km marker	GI
2072	*P. keeni*	Phantom Creek, 10km marker	GI
2073	*P. keeni*	Rennell Sound Rec. Site	GI
2074	*P. keeni*	Rennell Sound Rec. Site	GI
2075	*P. keeni*	Rennell Sound Rec. Site	GI
2076	*P. keeni*	Rennell Sound Rec. Site	GI
2112	*P. keeni*	3.5km E Masset	GI
2168	*P. keeni*	5 Mile Point	GI
2043	*P. keeni*	Gray Bay Recreation Site	MI
2044	*P. keeni*	Gray Bay Recreation Site	MI
2045	*P. keeni*	Gray Bay Recreation Site	MI
2046	*P. keeni*	Gray Bay Recreation Site	MI
2047	*P. keeni*	Gray Bay Recreation Site	MI
2048	*P. keeni*	Gray Bay Recreation Site	MI
2049	*P. keeni*	Gray Bay Recreation Site	MI
2050	*P. keeni*	Gray Bay Recreation Site	MI
2051	*P. keeni*	Gray Bay Recreation Site	MI
2052	*P. keeni*	Gray Bay Recreation Site	MI
2053	*P. keeni*	Gray Bay Recreation Site	MI
2056	*P. keeni*	Gray Bay Recreation Site	MI
2058	*P. keeni*	Gray Bay Recreation Site	MI
2060	*P. keeni*	Gray Bay Recreation Site	MI
2061	*P. keeni*	Gray Bay Recreation Site	MI
2062	*P. keeni*	Gray Bay Recreation Site	MI
2063	*P. keeni*	Gray Bay Recreation Site	MI

2064	*P. keeni*	Gray Bay Recreation Site	MI
2065	*P. keeni*	Gray Bay Recreation Site	MI
2066	*P. keeni*	Gray Bay Recreation Site	MI
6683	*P. keeni*	Triangle Island	TI
6682	*P. keeni*	Triangle Island	TI
6681	*P. keeni*	Triangle Island	TI
6680	*P. keeni*	Triangle Island	TI
6678	*P. keeni*	Triangle Island	TI
6677	*P. keeni*	Triangle Island	TI
6676	*P. keeni*	Triangle Island	TI
6675	*P. keeni*	Triangle Island	TI
6674	*P. keeni*	Triangle Island	TI
6673	*P. keeni*	Triangle Island	TI
6672	*P. keeni*	Triangle Island	TI
6671	*P. keeni*	Triangle Island	TI
6670	*P. keeni*	Triangle Island	TI
6669	*P. keeni*	Triangle Island	TI
6668	*P. keeni*	Triangle Island	TI
6667	*P. keeni*	Triangle Island	TI
6666	*P. keeni*	Triangle Island	TI
6665	*P. keeni*	Triangle Island	TI
6664	*P. keeni*	Triangle Island	TI
6663	*P. keeni*	Triangle Island	TI
6662	*P. keeni*	Triangle Island	TI
6661	*P. keeni*	Triangle Island	TI
6660	*P. keeni*	Triangle Island	TI
6659	*P. keeni*	Triangle Island	TI
6658	*P. keeni*	Triangle Island	TI
6657	*P. keeni*	Triangle Island	TI
6654	*P. keeni*	Triangle Island	TI

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EDUCATION:

Lamar University	B.S.	1999	Biology
Lamar University	M.S.	2002	Biology
Texas A&M University	Ph.D.	2005	Biology

RESEARCH INTERESTS:

Genetics, Vertebrate Systematics, Biodiversity and Evolutionary Biology

COURSES TAUGHT, UNDERGRADUATE:

General Biology I & II Laboratory, Desert Field Mammalogy, Desert Field Biology, Genetics Supplemental Instruction, Biomedical Genetics Recitation, Biomedical Genetics (Guest), Microbiology Laboratory, Chordate Anatomy Laboratory, Principles of Evolution (TA, Guest), Mammalogy (field portion), Introductory Biology Lecture, Conservation and Management (Guest)

COURSES TAUGHT, GRADUATE:

Methods of Teaching Biology Laboratory

MENTORSHIPS:

Graduate Mentor, Trainer of 10 undergraduate and 2 graduate research students
Graduate Mentor, NSF-REU (Summer 2004, 2005)
Mentor, Aggie Women in Leadership – Texas A&M University (2004 – 2005)

AWARDS:

Robert L. Packard Award, Texas Society of Mammalogists Meeting, 2004
2005 Biology Graduate Teaching Award ($1000), Department of Biology

PUBLICATIONS:

Walker, M. L. 2002. A molecular and chromosomal assessment of the consequences and extent of hybridization between *Crotalus atrox* and *C. scutulatus*. Lamar University, Beaumont, TX.

Submitted for review:

Walker, M. L., and M. W. Haiduk. 2005. Preliminary results concerning ostensive hybridization between *Crotalus atrox* and *C. scutulatus* (Viperidae). Submitted to The Southwestern Nat.

Walker, M. L., S. E. Chirhart, A. F. Moore, R. L. Honeycutt, and I. F. Greenbaum. 2005. Genealogical concordance and the specific status of *Peromyscus sejugis*. Submitted to J. Hered.